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STUDIES ON BOVINE MASTITIS.

V.—THE MORE ACUTE FORMS OF STREPTOCOCCUS MASTITIS.

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THE main purpose of the present article is to stress the existence of a form of streptococcus mastitis which runs an acute or subacute course and which is to be distinguished from the widely prevalent and well-recognised chronic variety. The bacteriology of this condition will only be referred to cursorily, since it is intended to deal fully with the matter in succeeding papers. It must be made clear, however, at the outset that the streptococci associated with the more acute changes differ in important respects from those which can be isolated from chronic mastitis.

The views of earlier authors regarding the diseased conditions of the cow's udder are set out in publications by Th. Kitt (1886, 1913), B. Bang (1889), C. O. Jensen (1897), Zschokke (1897, 1904), S. Wall (1908), and Ernst (1909, 1910). While deep-seated or " parenchymatous " inflammation of the gland was recognised to be chiefly due to such organisms as staphylococci, *B. coli*, or *B. pyogenes*, it was accepted that the most prevalent form of all—a chronic catarrhal form, spreading by contagion and first referred to as " *gelber Galt* "* by Swiss authors—was due to streptococci. In those early days when an attempt was made to draw distinctions between the various conditions, the chief emphasis was naturally laid upon such points as the course of the disease and the clinical symptoms shown by the animal. The route by which infective material was supposed to reach the gland and the

* The expression " *gelber Galt* " implies a condition in which the milk secretion is changed to a yellowish fluid and is greatly reduced in quantity.

manner in which the disease originated were also taken into consideration. In this way cases of mastitis were referred to as acute or chronic, curable or incurable, sporadic or infectious, lymphogenous, hæmatogenous or galactogenous (Kitt, 1886 ; Guillebeau and Hess, 1890 to 1894 ; Zschokke, 1897, 1904).

The early distinctions drawn between the different routes by which it was considered that infective material might be introduced into the udder have at least served to emphasise the belief, which is not improbably correct, that this route is usually the teat canal.

The first recognition of the importance of the bacterial species in influencing the clinical picture was largely due to the work of B. Bang (1889). At the same time it was recognised that cases that were similar clinically might be due to different varieties of bacteria. The view, at one time held by Zschokke (1897), that cases of "*gelber Galt*" could be distinguished as curable and incurable, that the former were associated with short streptococcus chains and the latter with long chains, was quickly abandoned, partly as a result of the work of Stäheli (1904). Similarly, the view that some cases of "*Galt*" were sporadic in origin and that these were to be distinguished from cases which arose by contagion is no longer accepted. It has often been remarked by clinicians that in mastitis the local symptoms are sometimes acute and may be accompanied by systemic disturbance, while at other times the disease is purely local and chronic from the outset. It has also been observed that many acute cases are associated with short-chained streptococci in the secretion. The tendency, however, has been to assume that clinical differences were due to such factors as the period of lactation at which infection took place or to the variable natural resistance of the animal. The extent to which such differences were dependent upon the kind or virulence of the streptococcus has been a matter for speculation.

In the absence of any really satisfactory methods for differentiating such organisms as streptococci from one another, it was clearly impossible to comprehend the real import of clinical observation. On the other hand, it may be put forward with much reason that a satisfactory classification of such infections must rest upon an ætiological basis. The first noteworthy efforts to define with greater precision the bacterial species responsible for mastitis were made by Savage (1906 to 1908), and these were extended by the work of F. S. Jones (1918), Klimmer, Haupt and Roots (1928), and others. It is now recognised that at least the majority of streptococcus strains which can be isolated from cases of mastitis belong to a fairly well-defined group, to which the name *S. mastitidis contagiosæ* (Nocard and Mollereau, 1887) has sometimes been applied in the past. According to Klimmer and Haupt (1930) the proper designation for this group should be *Streptococcus agalactiæ* (Kitt, 1893). Some strains coming within this category when growing in plates of ox blood agar form narrow beta-hæmolytic zones in the sense of J. H. Brown (1919), while others are alpha-hæmolytic. Purely for the sake of convenience, it is proposed in this paper to use the term *Streptococcus agalactiæ* when referring to this group.

The views which have been expressed by workers on the continent regarding the clinical and bacteriological aspects of streptococcus mastitis may be summarised as follows. In Germany, Klimmer, Haupt and Roots (1928), and Haupt (1931), state that it is only in isolated instances that mastitis is caused by streptococci other than *S. agalactiæ* or actively hæmolytic streptococci. One such example is discussed in the 1928 paper. It is stated (Klimmer and Haupt, 1930) that the chief difference between "*Galt*" and

other infections of the udder due to streptococci lies in the course of the disease. While with " *Galt* " spontaneous healing within a lactation period usually does not occur, infections with other (" vulgären ") streptococci tend to heal up as a rule within a few weeks. According to Seelemann (1928), the onset of mastitis is insidious in nearly every case, though there may be acute phases during the course of the disease. Up to 1928 he had met with no cases in which the causal streptococcus did not possess the properties described by him as characteristic of the true mastitis streptococcus (*i.e.*, *S. agalactiæ*). Seelemann reports, however, that he has rather frequently observed instances in which the milk secretion was physically and chemically altered, but from which bacteria recognised to be pathogenic could not at the time be isolated. From a few of these *S. agalactiæ* was eventually grown, but from the majority either no streptococci at all were cultivated or streptococci which were different from *S. agalactiæ* and which were not looked upon as causally connected with the condition. Instances such as these were, therefore, referred to as " non-specific disturbances " of the udder. It is apparent, however, from a monograph, which has just been published (1932), that in the light of further experience Seelemann has to some extent revised his views. Apart from mastitis due to *S. agalactiæ* and to actively hæmolytic streptococci, this author has now seen in a number of herds infections due to streptococci closely resembling *S. agalactiæ* but described as " atypical " on account of slight cultural differences. These infections could not be distinguished clinically from the chronic form of mastitis due to the typical *S. agalactiæ*, but they appeared to arise sporadically and to be connected with recent outbreaks of foot-and-mouth disease or with wounds about the teats. Finally, on a few occasions Seelemann has found still other kinds of streptococci in mastitis, but no description is given of the clinical symptoms shown.

In Austria, Diernhofer (1930) has quite definitely concluded that streptococci other than *S. agalactiæ* and actively hæmolytic streptococci can cause acute mastitis in cows with atrophy of the gland, although such occurrences are comparatively rare. Instances of this sort are described in his paper. As the animals in question had recently passed through an attack of foot-and-mouth disease, it was considered doubtful whether such uncommon streptococci could cause outbreaks of mastitis in healthy herds.

Our earlier investigations (1929) also led us to conclude that streptococci possessing characters other than those of *S. agalactiæ* or actively hæmolytic streptococci might be associated at times with pathological disturbance of the gland, and this view has been reiterated in an article recently published by us in the *Zentralblatt für Bakteriologie* (1931). In this and immediately succeeding papers it is desired to bring forward further evidence on the point.

During the past three years we have been interested in the control of mastitis, our efforts having been chiefly directed against the chronic form of the disease, caused by *S. agalactiæ*. It does not fall within the scope of this paper to refer to this work at length. In one of the herds under control—Herd A, to which most of the observations described in this paper relate—no udder disturbances due to *S. agalactiæ* have been observed. The original herd of some 50 milking cows contained eight which were proved to be infected with this organism, and these were promptly eliminated. For the past two and a half years milk samples from the individual quarters of every cow in milk have been examined by plating the centrifugal deposits in blood agar and in

other ways. This has been done as a routine at three-monthly intervals, and in addition milk samples from all cows six to ten days after calving and from all cows showing abnormalities in the udder or milk have been examined in a similar manner.

Except in two doubtful cases, streptococci with the characters of *S. agalactiæ* have not been encountered. In Herd B only two or three of the cows originally infected with *S. agalactiæ* now remain, and these are kept well away from the main herd and are used solely for breeding and for rearing bull calves. In Herds C and D a number of the cows originally infected with *S. agalactiæ* still form part of the milking herd. Since July, 1929, the infected cows have been milked apart, and among those originally regarded as healthy only two or three instances in each herd of *S. agalactiæ* infection have appeared.

In Herd A the eradication of *S. agalactiæ* has by no means brought about the disappearance of streptococcus mastitis, and the same is true to a smaller extent of Herd B and of the " healthy " sections of Herds C and D. As stated previously, bacteriological and serological details are reserved for later papers, but it must be emphasised here that the characters of the streptococci isolated from the secretion and which there is strong reason to believe were the cause of the mastitis were clearly different from those of *S. agalactiæ* For the most part, the strains isolated belonged to the group referred to as " non-hæmolytic group II " in our paper published in 1929.

Herd A. Further History and Clinical Symptoms Shown by Affected Animals.

At the beginning of 1930, 1931 and 1932 the milking herd consisted of 55 (45 Shorthorn, ten Ayrshire) ; 71 (two Shorthorn, 69 Ayrshire) and 113 (two Shorthorn, 111 Ayrshire) cows respectively. Thus, the great majority of the Shorthorns were disposed of during 1930, and since the middle of 1929 the herd has been built up by the purchase of first-calving Ayrshire heifers. The ages of the animals present during 1930 could not be determined, but at the beginning of 1931 and 1932 the mean ages were 3·4 years and 4 years, the corresponding modes being approximately 3·2 and 3·8 years. The herd is free from tuberculosis. Contagious abortion is kept well under control by three-monthly agglutination tests and by isolation or disposal of the few animals reacting. Milking is carried out by machine thrice daily, the yield of each animal being recorded at each milking. We have been fortunate in that the owner, a thorough business man and a keen observer, has immediately brought to our notice any abnormalities in the milk yield or the condition of the udder, no matter how slight or transitory these might be. As explained earlier, in addition to routine milk sampling, udder secretion from all such cows has been immediately forwarded to the Institute for examination.

The severity of the symptoms and the amount of permanent damage to the milk secreting tissue varied from case to case, and the following general account is compiled from the observations of the owner and of ourselves.

There has been no pronounced systemic disturbance. Local symptoms were usually confined to one quarter of the udder, though not to any particular quarter. The onset was usually sudden, a quarter which was normal at the previous milking being found to be uniformly and tensely swollen and painful. The swelling might also involve the teat, and at this stage it was often difficult to obtain any secretion. The owner was quite definite that a prognostic sign in many cases was the presence of scabby material at the orifice of the teat and this was followed two or three days later by swelling of the quarter. It has been found necessary at times to remove this scab before the cow could be milked.

In the most severe cases the acute symptoms lasted three or four days, after which the swelling subsided but the milk secretion failed to reappear and the quarter gradually became atrophied and indurated. Sometimes the destructive changes were so pronounced that the quarter practically disappeared. In less severe cases acute swelling of the quarter, accompanied by almost complete cessation of the milk yield, was followed by a return to practically normal conditions after a few days. Some " weakness " of the quarter, however, usually remained, and localised masses of induration could afterwards be detected on palpation. In some instances, similar symptoms have recurred in the same quarter several times at intervals of a few days.

A number of mild cases have been seen, in which slight and transient swelling of a quarter, accompanied by considerable fall in the milk yield, has been followed in less than 48 hours by a return to normal conditions. No permanent damage to the udder could afterwards be appreciated on manipulation. Whether the mild course of these cases was due to prompt and repeated stripping of the affected quarter could not be determined. In the more severe cases the characters of the secretion were visibly altered to a greyish or yellowish liquid containing clots. In mild cases there was often no naked-eye change in the milk but laboratory examination showed abnormal alkalinity and increased cell content.

Between November, 1929, and September, 1931, 24 cases of more or less acute mastitis with symptoms of the above nature have been observed in Herd A. Twelve of these cases occurred during the first year of this period. Twelve of the 24 cases were severe and were followed by considerable permanent damage to or complete loss of the quarter. With the remainder the ultimate damage was less extensive and milk secretion returned to its former level or was only slightly reduced. Six cases were extremely mild.

From all these 24 cases non-hæmolytic streptococci with characters other than those of *S. agalactiæ* were isolated, usually in large numbers, and other pathogenic bacteria were absent. In most

instances, bacteriological examination, carried out on several occasions, and at times only a few days prior to the attack, had shown the quarter to be normal. In mild cases the streptococci disappeared as soon as the symptoms subsided. In more severe cases they were excreted for a longer period, but there was less tendency for permanent infection than is shown by *S. agalactiæ*. In two other instances similar streptococci were isolated from the apparently normal milk of cows which showed no symptoms of mastitis. Finally, there were four cases where cows had shown mild symptoms as above described but from which no streptococci were isolated, or only streptococci regarded as saprophytic in nature.

Of the 24 cases, seven occurred in Shorthorns and 17 in Ayrshires ; eight cases were in first calvers, four in second calvers, and two in third calvers. The condition was not confined to any particular stage of the lactation period. Thirteen of the cases occurred between October and March, and eleven between April and September. Immediately a cow was noticed to be showing symptoms of mastitis it has been the invariable practice to milk her separately. In spite of this precaution the disease continued to appear at irregular intervals and in circumstances where there was no possibility of infection being spread by milking or by direct contact with previous cases. Hence there are good grounds for believing that the disease arose sporadically.

The accompanying Charts 1 to 5 illustrate the effect of this form of mastitis upon the milk yield. It is considered unnecessary to reproduce the daily milk yields over the full lactation period of these animals, because prior to the attack the variations could not be considered abnormal. Charts 1 and 2, prepared from the lactation records of two severe cases followed by total loss of the affected quarter, show a permanent daily reduction of seven to eleven pounds of milk, *i.e.*, one-fifth to one-third of the yield. Charts 3 and 4 are compiled from the records of cases of medium severity, in which after the acute stage the daily yield was slightly reduced. Chart 5 illustrates the temporary disturbing effect witnessed in mild cases.

Further History of Herds B, C and D.

The number of animals of milking age in these three herds is now as follows :—Herd B 27 (red poll), Herd C 92 (Ayrshire, red poll, and Jersey), and Herd D 50 (Shorthorn). The cows are milked by hand. The herds are free from tuberculosis ; Herd B is free from contagious abortion, and in Herds C and D this disease has not been a source of trouble for a number of years.

Since April, 1930, eight definite cases of mastitis due to non-hæmolytic streptococci other than *S. agalactiæ* have occurred in these three herds. Of these cases, two occurred in Herd B, and four and two respectively among the " healthy " animals in Herds C and D. The clinical symptoms corresponded with those seen in Herd A, but on the whole were not severe.

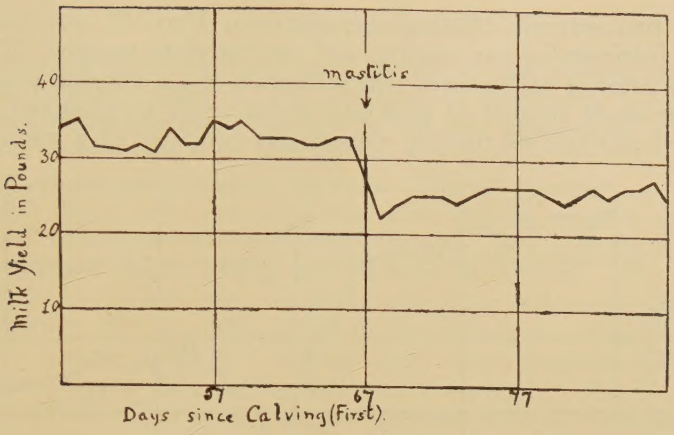

CHART 1.
Cow 'Plum'. Severe mastitis left fore quarter.

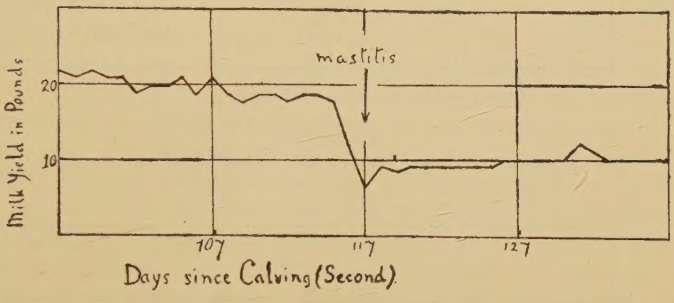

CHART 2.
Cow 'Queenie'. Severe mastitis left hind quarter.

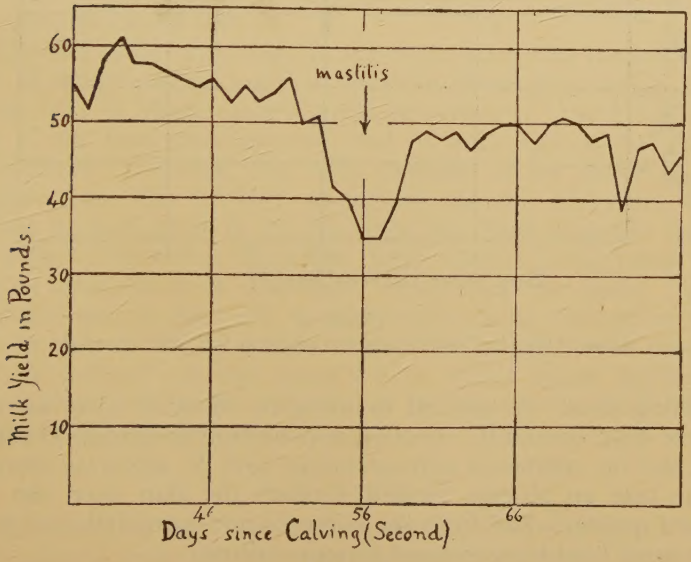

CHART 3.
Cow 'A. Fanny'. Mastitis of moderate severity left fore quarter.

Of two severely affected animals from Herd C, one was kept at the Institute for two months and one for eight months. The left hind quarter of each animal was indurated and somewhat atrophied, and the small amount of secretion which could be obtained during the first two and six months respectively consisted of a watery fluid

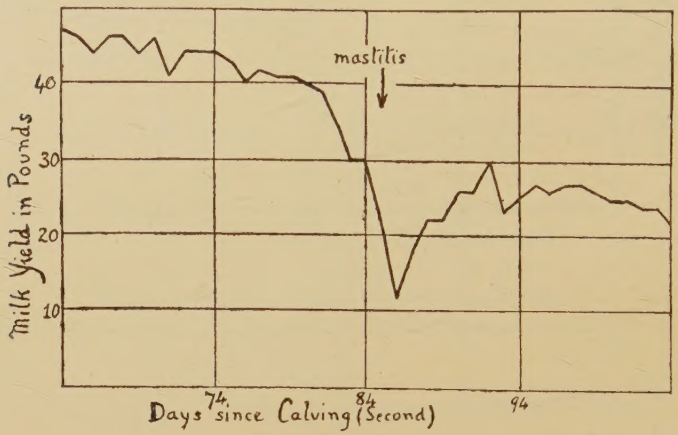

CHART 4
Cow 'Lenny'. Moderately severe mastitis left fore quarter,

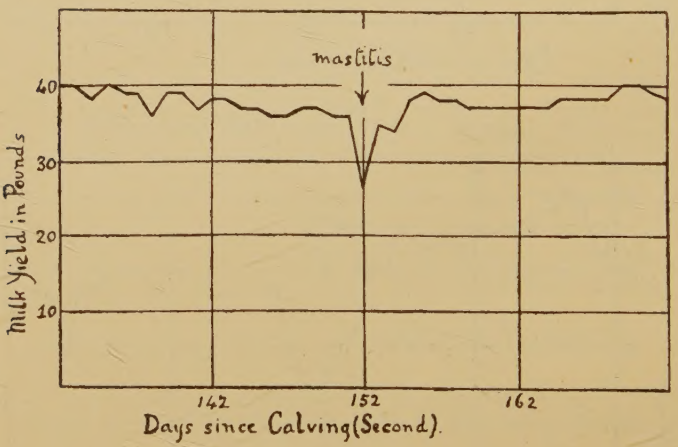

CHART 5.
Cow 'Hope'. Mild transient mastitis left hind quarter.

containing clots. A cultural examination made at intervals of two or three days constantly revealed non-hæmolytic (group II) streptococci, but no organisms corresponding with *S. agalactiæ* were seen. In one case an abscess formed beneath the skin over one of the diseased quarters, and from the contents non-hæmolytic streptococci of the same kind were isolated in pure culture.

Discussion.

Although a good deal has been written on the subject of streptococcus mastitis, there is still evidence of confusion between the different clinical forms. Even to-day the real significance of the distinction between the more acute forms and those which are essentially chronic is not generally recognised. This is due primarily to the fact that distinctions are often based on clinical observations alone and such differences are not always clear-cut. It is acknowledged that acute phases occur during the course of chronic streptococcus mastitis ; in fact as a rule this is the only stage seen by the practising veterinarian. On the other hand, in the more acute form, where the affected quarter does not undergo early and complete destruction, the later stages tend to be somewhat chronic. Fundamentally, however, the confusion between the different clinical forms has been due to the bacteriology of mammary diseases not having been fully worked out.

In the past, attention has been almost entirely confined to the chronic streptococcus infections of the udder, largely on account of their greater frequency and economic importance. Further, in some laboratories, especially in Germany, culture methods have been introduced which are selective for the streptococcus of chronic mastitis, and this procedure in our view has tended to obscure the existence of less common pathogenic streptococci. Nevertheless, there are obvious reasons why the streptococcus diseases of the udder should be clearly differentiated from one another. It is impossible to introduce thoroughly sound methods of control, whether by hygienic measures or by some system of vaccination, until full information is available regarding the bacteriology of these affections, and until this information has been studied in relation to clinical and epizootiological data.

The observations recorded in this paper show that the less common form of streptococcus mastitis, in spite of its presumably sporadic nature, may at times assume great importance. Herd A, in which most of the data were secured, was of quite exceptional value for observational purposes, not only because of its almost complete freedom from the ordinary contagious diseases of cattle, but also because no indication of *S. agalactiæ* has been observed for about two years. There has, therefore, been a clear background for the study of other forms of mastitis to which cattle are liable. While it is possible that in this herd special predisposing influences were at work—which at the moment it would be premature to discuss—it must be pointed out that mastitis of a similar nature has been met with in other herds. An account of our experience with three of these herds has been given above.

Summary.

The views of previous workers who have investigated mastitis in its clinical and bacteriological aspects have been discussed.

The paper stresses the existence of a form of streptococcus mastitis which commonly runs an acute course and is to be distinguished from the widely prevalent chronic form. An instance is described of a herd, now numbering some 110 cows, in which there occurred over a space of two years 24 cases of the more acute form. Twelve of these were of such severity that the affected quarter was partially or completely destroyed. With such animals there was a permanent reduction in milk yield, estimated to range from seven to eleven pounds daily, or one-fifth to one-third of the supply. The value of this herd for observational purposes was increased by the fact that for about two years udder disturbances due to *S. agalactiæ* have not been observed. The disease appeared to arise spontaneously, and there was no obvious relationship to breed, age, stage of lactation, or season of the year. For reasons which are not at present clear, the disease in this herd assumed great importance owing to its relatively high incidence and the severity of some of the cases. The acute form of the disease has been met with, however, in other herds and an account is given of our experience in three of these.

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STUDIES ON FELINE DISTEMPER.

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Introduction.

The diseases of the cat have hitherto received comparatively little attention, although there is no animal so universally kept in houses as the ordinary domestic cat.

Our attention was first directed to this subject by Dr. Vevers and Col. Hamerton, who mentioned the occurrence of very fatal epidemics among the " lesser cats " in the Gardens of the Zoological Society of London. Through their kindness we were able to examine a number of animals that had died in the Gardens, and from the symptoms and post-mortem appearances the infection was diagnosed as the abdominal form of feline distemper. Subsequently attention was directed to the epidemics of " Show Fever," which have become such a serious problem to cat breeders in this country.

The institution of cat shows, whereby animals from widely divergent localities are brought together, furnishes ideal conditions for the spread of this highly contagious disease, and, as a result, most breeders sooner or later suffer from epidemics. Siamese and Persian varieties are particularly susceptible, especially kittens, but all cats, no matter what their breed or age, are liable to be attacked. Through the very kind assistance of veterinarians and cat fanciers, we have had opportunities both of studying epidemics that have occurred after shows and of making post-mortem examinations of numerous animals that have succumbed to the disease.

With the object of determining the nature of feline distemper, which in many respects shows a remarkable similarity both to canine distemper and to human influenza, we have made careful pathological and bacteriological examinations of the material at our disposal, and also made experiments to determine the nature of the infection, with a view to finding out more efficient methods of prevention and control.

In the absence of a supply of kittens bred under conditions excluding the possibility of an exposure to chance infection, our results are still incomplete, but the following preliminary observations are being published in the hope of throwing further light on the ætiology of this and allied conditions.

Historical.

It is generally agreed that feline distemper, like canine distemper, may show protean symptoms, and in the absence of any definite ætiological agent there is often difficulty in determining the nature of any particular case. Hamilton Kirk (1925), in his excellent treatise on the Diseases of the Cat, refers to the variety of symptoms manifested by distemper, and classifies them broadly as catarrhal, pharyngeal, pulmonary, and abdominal.

The pathological investigations that have hitherto been recorded do little towards solving the problem, for, in view of the results of our experiments, we are convinced that the records of various micro-organisms that have been isolated from cats dying of epidemic diseases refer in many cases to secondary invading organisms, and not to the primary cause of the disease. Cats, especially when young, seem to be peculiarly susceptible to the invasion of secondary organisms, usually from the intestinal tract, as is well shown in the case of animals infected with *Endamœba histolytica*, which almost invariably succumb to a septicæmia.

The only authors to publish any observations suggesting that this disease might be due to a filterable virus are Verge and Cristofroni (1928), who inoculated one cat with the filtrate of the organs of a Siamese cat which had died of " infectious gastro-enteritis." The inoculated cat died eleven days later with " typical " symptoms, and in turn a filtrate of its organs was inoculated into a second animal which showed slight illness between the fifteenth and eighteenth days, but recovered.

Pathological Changes.

The material that we have studied may be divided into two groups : imported Felidæ dying at the London Zoological Gardens, and various kinds of domestic cats, mainly Siamese and Persian, infected for the most part at cat shows.

The disease has been seen in the following species of wild cats : American Tiger Cat, *Felis tigrina* ; African Tiger Cat. *F. aurata* ; Rusty Tiger Cat, *F. planiceps* ; Marbled Cat, *F. marmorata* ; Caracal lynx, *F. caracal* ; Ocelot, *F. pardalis* ; and the leopard, *F. pardus*. The pathological changes found in wild animals and numerous domestic cats dying of this disease consist typically of catarrhal congestion of the mucosa of some part of the small intestine. This congestion ranges from a few small patches in the ileum to involvement of the whole length of the small intestine, and exceptionally may extend into the large intestine and the stomach. Altered blood was occasionally found in the stomach and intestine. The mesenteric and posterior lymphatic glands were often enlarged and congested, and occasionally also the cervical lymphatics. Congestion of the lungs, enlargement of the spleen, pleurisy with effusion, peritonitis, and in three cases a hæmorrhagic thymus, were other changes noticed.

Histology.

Microscopical examination of the *intestine* has shown changes varying from simple congestion of the vessels in the submucosa, to hæmorrhage, sloughing, and destruction of the mucous membrane. Occasionally there were also small hæmorrhages in the muscular layers. In extreme cases the mucosa was covered by a fibrinous deposit including red cells, desquamated epithelium, and bacteria. When examined at an early stage, however, congestion of the capillaries in the submucosa was the only obvious change, and there was no desquamation of the epithelium. Similar changes were also observed in the few cases in which the congestion had involved the large intestine. Infiltration with polymorphonuclears was not a prominent feature in the lesions in the mucosa and submucosa.

The *lymphatic glands* often show hæmorrhages, principally in the cortex, which is usually congested. Occasionally actual hæmorrhages are absent, but the sinuses contain large numbers of red cells and polymorphonuclear leucocytes in various stages of degeneration.

The *lungs* were examined in specimens showing obvious naked-eye changes. In some cases there was congestion of the inter-alveolar capillaries, which occasionally were ruptured, the alveoli becoming filled with red cells. Sometimes the alveoli contained fibrinous exudate together with a few mononuclear cells. The exudate was occasionally present in the bronchi, which contained fibrin but no polymorphonuclear cells. The lining of the bronchi remained intact.

The *trachea* was very rarely involved and then only to the extent of some congestion of the blood vessels in the submucosa, without any definite evidence of inflammatory changes.

The *thymus* in three cases was the site of extensive hæmorrhage ; these animals all showed a streptococcal septicæmia, which was doubtless responsible for these changes.

The *spleen* showed no definite changes beyond slight congestion and phagocytosis of red cells.

The *liver* and *kidneys* showed no definite pathological changes beyond cloudy swelling.

Clinical Symptoms.

The clinical symptoms have been extremely vague and in many cases animals have died with no definite symptoms. In others, however, there has been diarrhœa and running of the eyes and nose. Fever is often present, ranging from 103° to 105° F., but many animals have died with typical pathological changes without showing any significant rise in temperature.

Bacteriological Examinations.

These have been made on 300 cases of the disease. In 8 per cent. no organisms could be isolated, whilst in all the others there was

evidence of bacteriological contamination. The following were the chief types encountered.

(1) *Bacillus coli.*
(2) A motile Gram-negative bacillus of the *Salmonella* group with many affinities to *B. typhi murium.*
(3) Hæmolytic Streptococci.
(4) *B. bronchisepticus.*
(5) *Pasteurella.*
(6) Hæmophilic Bacteria.
(7) *Streptothrix.*
(8) *Staphylococci.*

Bacteriological examinations failed to show the constant presence of any one type of organism, and the fact that certain cases of feline distemper were bacteriologically sterile is suggestive evidence that bacteria are not the primary cause of the disease.

Experimental Investigations.

Although the infectious nature of feline distemper is generally recognised, we have been unable to find any definite experimental evidence on this subject. The common occurrence of outbreaks after cat shows is, however, well known, and any illness developing one day to a month after such shows is commonly attributed to infection acquired in this manner. In the following experiments we have attempted to throw some light on the nature of the infection and also on the way in which it may be conveyed to other animals.

Infection by Fomites.

There is abundant evidence to show that feline distemper can be acquired by cats that have never been in direct contact with infected animals. Thus both the domestic house cats belonging to ourselves became infected shortly after starting our investigations. In one case the infection may have been introduced on the clothing or shoes, as no infected animals had entered the house. In the other case some possibly infected kittens were kept isolated in a room of the house for two days before being removed elsewhere. After their removal the house cat came in contact with a tray of sawdust used by the kittens, and in turn became infected ten to twelve days later. Both these cats were adult animals, and in each case the disease merely produced fever up to 105° F., with loss of appetite and diarrhœa. Broth cultures made from the blood of one of these cats during the febrile period remained sterile.

The following experiments also show that infection can be spread by fomites.

Experiment No. 31. December 5th, 1930.—An eight weeks old kitten was placed in a cage with a blanket used the previous day by a Siamese cat that had died of the disease. This kitten became infected and died nine days later, without showing any marked febrile symptoms.

Experiment No. 52. December 10th, 1930.—A six weeks old kitten, placed in a cage with the same blanket as that used in the foregoing experiment.

This kitten had a sudden rise in temperature on the ninth day, and was then killed. Post-mortem examination showed typical congestion of the small intestine.

Experiment No. 157. November 9th, 1931.—Two kittens, each about eight weeks old, were placed on a blanket that, after use by an infected Siamese cat, had been kept in a box at room temperature for 16 days. Both these animals became infected and died with typical post-mortem appearances, respectively seven and eleven days after being exposed to infection.

Infectivity of Naso-Pharyngeal Secretions.

With the object of determining the various ways in which this disease is spread, experiments were made with saline washings from the naso-pharynx of infected animals. These washings, after being passed through a Berkefeld N filter, were either sprayed into the nasal passages of normal kittens, or, in some cases, inoculated either subcutaneously or intraperitoneally. The results of these experiments show that the infective agent is present in nasopharyngeal washings, and also is capable of passing through a Berkefeld N filter.

Experiment No. 57. December 13th, 1930.—Two kittens, each about eight weeks old, were sprayed in the nasal cavities with a Berkefeld filtrate of a saline suspension of the nasal washings from a Siamese cat that had died of feline distemper. The first of these kittens showed a well marked rise in temperature seven days later, and was killed and examined. The small intestine was congested along its whole length, and there was slight but distinct congestion on the dorsal surface of the lungs. Broth cultures of the blood, lung, spleen, and cervical and mesenteric lymphatic glands all remained sterile.

The second kitten, similarly inoculated and kept in the same cage, showed a well marked rise in temperature on the eighth day and the fever continued for three days, the animal being found dead on the eleventh day. This kitten showed much more advanced symptoms than the first, there being a general peritonitis, with fibrinous exudate, in addition to the general congestion of the small intestine and enlargement of the mesenteric lymphatic glands. Cultures of the blood contained a *Streptococcus*, and the peritoneal exudate contained a *Streptococcus* and a coliform organism.

This seems to be a good example of the general course of the disease, as in the case of the kitten killed on the first day of fever the characteristic congestion of the small intestine had developed but all the tissues were sterile. When the fever had continued longer, as in the second kitten, secondary infections had become superimposed on the primary one, and in addition to the pathological changes bacteria had invaded the blood.

Experiment No. 83.—Three kittens, each about five to six weeks old, were infected from the first kitten of the foregoing experiment (No. 57). Two kittens were sprayed with filtered nasal washings collected on the first day of fever, and the third was inoculated intraperitoneally with a filtrate of the spleen and mesenteric lymphatic glands of the same animal. One of the " sprayed " kittens was killed on the eighth day, when its temperature was 104·8° F. The lungs showed signs of congestion and the peritoneal

cavity contained an exudate with an accumulation of pus on the floor of the pelvis. The small intestine was slightly congested and the mesenteric lymphatic glands enlarged. The blood contained a *Streptococcus*.

The other "sprayed" kitten died on the eleventh day and showed similar though more advanced pathological change. A *Streptococcus* was found in the cultures of the blood, mixed with an organism giving the reactions of the *Salmonella* group in the peritoneal exudate. The lung and trachea also contained a mixture of organisms.

The third kitten, infected by intraperitoneal inoculation of a filtrate of the organs, showed conjunctivitis accompanied by fever on the eleventh day and was killed *in extremis* on the sixteenth day. The blood, lung, spleen, and mesenteric glands all contained a strain of *Salmonella*.

The above experiments are selected from among many others all leading to the same conclusion, namely, the infectious nature of the naso-pharyngeal washings even after passing through a Berkefeld filter. Consequently it seems probable that the infection is usually spread by means of infective material produced by coughing. This would explain the way in which cats may become infected without coming in direct contact with other diseased animals.

Studies of Infectivity of Cats from Various Epidemics.

In view of the variety of symptoms, as well as the differences in the post-mortem findings, it seemed of importance to study the types of infection produced in normal cats by the inoculation of infected material from different sources. Consequently, we have made a series of passages from various strains, the results of which give some indication of the types of infection produced.

It is only possible to give a few examples of these passages and, therefore, we have selected three typical strains :—

Strain Siamese V.

The original source of this strain was an adult Siamese cat which died, along with others, during a typical epidemic of feline distemper in a large cattery. Cultures of the heart blood and mesenteric lymphatic glands of the animal were sterile.

Six passages were made with this strain, the results of which are shown in the table on page 17.

It will be seen that only two kittens survived out of a total of fourteen. Excluding Cat No. 64 which was killed and No. 44, which survived for 21 days, the average duration of the infection is approximately eight days, and there is no evidence of any diminution in the virulence of the strain during these six passages. The variety of secondary invading organisms is well exemplified in this series.

Strain Siamese XI.

The original source of this strain was also a Siamese cat which presumably had acquired the infection at a show, as it became ill six days later and died after two days' illness. The blood, cervical lymph

STRAIN SIAMESE V.

No. of Cat.	*Inoculum.*	*Duration of Disease.*	*Result of Cultures of* *Blood.*	*Lung.*	*Spleen.*	*Mes.*	*Remarks.*
1st passage							
43	Filtered organs of	9 days	Sterile	Contam.	Sterile	Sterile	
44	Siamese V.	21 days	Strep. & coliform	Various	Strep. & coliform	Strep. & coliform	
2nd passage							
63	Filtered organs of	Survived					
64	Cat 43	3 days (killed)	Sterile	Sterile	Strep.	Sterile	
3rd passage							
73	Filtered organs of	9 days (killed)	Strep.	Sterile	Strep.	Salmonella	
74	Cat 64	10 days	Salmonella	Various	Salmonella	Salmonella	
4th passage							
90	Filtered organs of	6 days	Strep.	Strep.	Strep.	Strep.	Peritoneal exudate containing Strep. & Pasteurella.
91	Cat 73	6 days	Strep. & Pasteurella	Ditto.			
5th passage							
106	Filtered nasal washings of Cats 90 & 91. 106 & 108 inoculated; 107 & 109 sprayed.	7 days	Strep.	Strep.	Strep.	Strep.	
107		12 days	Strep. & coliform	Strep.			Per.exudate Strep. & coliform
108		5 days	Sterile	Pneumo. & coliform	Coliform	Coliform	
109		6 days	Strep.	,,			Per.exudate Strep.
6th passage							
128	Sprayed with nasal washings of Cat 109	9 days	Strep. & Salmonella	Salmonella			Per.exudate Strep. & Salmonella
129		Survived					

gland, and spleen were sterile, but the lung contained a *Streptococcus* and a *Pasteurella*, while the mesenteric lymphatic gland also contained a *Pasteurella*.

Two kittens, Nos. 55 and 56, were inoculated intraperitoneally with a filtrate of the spleen and mesenteric glands, and another two, Nos. 57 and 58, were sprayed with a filtered suspension of the nasal washings. The results of this and subsequent passages are given in the following table :—

STRAIN SIAMESE XI.

No. of Cat.	*Inoculum.*	*Duration of Disease.*	*Result of Cultures of* *Blood.*	*Lung.*	*Spleen.*	*Mes.*	*Remarks.*
1st passage							
55	Filtered organs of Siamese XI Intraper.	29 days	Strep. & coliform	Ditto.	Ditto.	Ditto.	
56		12 days	Sterile	Various	Coliform	Coliform	
57	Sprayed with nasal washings of Siamese XI	7 days	Sterile	Sterile	Sterile	Sterile	
58		11 days	Strep.				Per.exudate Strep. & coliform.
2nd passage							
83	Sprayed with nasal washings of Cat 57	8 days	Strep.	Strep.	Strep.	Strep.	
84		12 days	Strep.	Various			Per.exudate Strep. &
85	Filtered organ of 57 intraper.	17 days	Salmonella	Salmonella	Salmonella	Salmonella	Salmonella
3rd passage							
92	Filtered nasal washings of Cat 83 Intraper.	33 days	Sterile	Strep.	Strep. & Ent.	Strep. & Ent.	
93	Sprayed in nasal cavity from Cat 83	19 days	Strep.	Strep.	?Subtilis	?Subtilis	
94		15 days	Strep.	Coliform	Strep. & coliform	Ditto.	
122	Sprayed with filtered nasal washingof Cat85	36 days	Strep.	Strep.	Strep.	Strep.	
123	Inoculated Intraper. with same.	20 days	Sterile	Sterile	Coliform	Coliform & subtilis	
124	Sprayed with filtered organs of Cat 85.	12 days	Sterile	Salmonella	Strep.	Salmonella	
125	Inoculated intraper. with same.	5 days	Strep.	Strep.			Per.exudate Strep.
4th passage							
117	Sprayed with nasal washings of 124.	Survived	but showed fever for some days				Blood cultures sterile
119		Survived	but showed fever for some days				„

The results (see p. 18) agree in the main with those obtained in the case of the Siamese Strain V, but the average duration of the infection is considerably longer. Only two kittens survived out of sixteen and both these had been inoculated intraperitoneally with dried virus twelve days before being sprayed with the filtered nasal washings of Cat 124. It is possible that they may have been protected by this inoculation, as the virus is either attenuated or killed by drying at ordinary room temperature *in vacuo*.

Both of them showed a well marked febrile reaction, respectively 15 and 18 days after being sprayed with the filtrate of Cat 124. Subsequently they have been repeatedly re-inoculated with virus without showing any reaction.

Siamese Strain IX.

This strain was obtained from a Siamese cat that also became infected at a show, but recovered after a severe illness. Blood was collected from the animal during the febrile stage and used for inoculation. Broth cultures of the blood remained sterile.

The results obtained with this strain are given in the following table :—

No. of Cat.	*Inoculum.*	*Duration of Disease.*	*Result of Cultivation of*				*Remarks.*
			Blood.	*Lung.*	*Spleen.*	*Mes.*	
1st passage							
45	Unfiltered blood of Siamese IX intraper.	32 days	Sterile	Coliform	Coliform	Coliform	
46	Filtered blood of Siamese IX intraper.	30 days	Salmonella & Strep.	Various			Per. exudate various organisms.
47		11 days	Salmonella	Salmonella	Salmonella	Salmonella	
2nd passage							
69	Filtered organs of Cat 47 Intraper.	19 days	—	—	—	—	Pleural exudate. Various organisms.
70		21 days	Sterile	Sterile	Sterile	Sterile	
3rd passage							
126	Filtered organs of Cat 70 Intraper.	16 days	Sterile	Sterile	Sterile	Coliform	
127		Survived	—	—	—	—	

Subcutaneous and Intraperitoneal Inoculations.

The great majority of our animals were infected by the subcutaneous or intraperitoneal inoculation of a suspension of spleen and mesenteric lymphatic glands after passage through a Berkefeld filter. The organs were ground up in either physiological saline

solution or broth, and the suspension centrifuged to remove larger particles before filtration. As might have been expected, animals inoculated in this manner regularly became infected, although, as with other methods of infection, considerable variations were observed in the course of the resulting disease.

Daily temperatures were taken of all our cats, but the results show that no reliance can be placed on this as a method of detecting infection, for, out of 55 animals inoculated either subcutaneously or intraperitoneally with virus, 34 showed no significant rise in temperature, including five out of the seven which survived. Consequently it is not possible to indicate the incubation period, as in many instances the cats died without showing any signs of illness, either febrile or otherwise. Out of 21 of these cats showing fever the average incubation period was between nine and ten days. The average duration between inoculation and the death of the animals in 46 animals was approximately 13 days, but there were considerable individual variations, probably depending to some extent on the type of secondary infection.

Intracerebral Inoculation.

It is well known that certain virus infections will produce encephalitis when inoculated into the brain (*e.g.*, herpes, vaccinia, yellow fever in mice, &c.) and it seemed of interest to determine whether the virus of feline distemper would have a similar effect.

Two kittens, Nos. 301 and 302, were inoculated intracerebrally with a Berkefeld filtrate of the brain of a cat dead of distemper. One of them (302) was killed *in extremis* three days later, and the other (301) showed a rise in temperature and was killed the following day. Both showed congestion of the small intestine, but no signs of encephalitis either clinically or on examination of the brain histologically.

Two kittens, Nos. 303 and 304, were each inoculated intracerebrally with a filtrate of the brain of No. 301. No. 303 died on the seventh day and showed marked congestion of the small intestine ; No. 304 had a rise in temperature the same day and was killed. The small intestine was slightly congested. Cultures of the blood, lungs, spleen, and mesenteric lymph glands remained sterile. Histologically neither of the animals showed any signs of encephalitis.

Two kittens, Nos. 305 and 306, were each inoculated with a filtered suspension of brain material from Kittens 303 and 304. Kitten No. 305 died on the twelfth day ; its small intestine was heavily congested for a length of about 10 in. towards the hinder end, and the mesenteric lymph glands were congested. Cultures of the organs were contaminated. No. 306 became ill about the seventh day, but after six days illness eventually recovered.

The results of these few experiments seem to indicate that the virus of feline distemper has no neurotropic affinities, but merely produces a generalised infection when inoculated intracerebrally.

Attempts to Infect Other Species of Animals.

Observations at the Prosectorium of the Zoological Society of London, by Col. Hamerton and others, indicate the susceptibility of

various species of cats belonging to the family Felidæ. Up to the present there seem to be no records of any of the larger members of this family, such as lions, tigers, and pumas, being affected, although young leopards are particularly susceptible.

The following experiments were made with other species of animals :—

Dogs.—There seems to be a widespread belief that dogs are susceptible to feline distemper, but we have been unable to find any evidence in support of this view. Laidlaw and Dunkin (1926, a) found that cats are not susceptible to canine distemper and our experiments with ferrets recorded below confirm their view as to the distinctness of these two diseases. We can find no record of dogs having been definitely exposed to infection with feline distemper, and, therefore, two puppies about four months old were each inoculated with a suspension of virus from a cat dead of the disease. Neither of the puppies showed any signs of infection, whilst two control kittens inoculated with the same suspension became infected and died of the disease.

Ferrets.—In view of the high susceptibility of this species to canine distemper, attempts were made to infect ferrets with the feline disease. Two animals were inoculated intraperitoneally with a Berkefeld filtrate of the spleen and lymphatics of cats dead of feline distemper, and in addition one of them was sprayed with unfiltered material. Neither of the ferrets showed any signs of infection during the two months they were under observation.

Mongoose.—Through the kindness of Major Dunkin and Dr. Laidlaw we were able to test the susceptibility of three mongooses. Each animal was inoculated intraperitoneally with a suspension of virus, but showed no signs of infection.

Rabbit.—Eleven rabbits were inoculated intraperitoneally at various times with virus but, with the exception of one individual that died of an intercurrent infection, none of them showed any febrile or other pathological symptoms.

Guinea-pig.—Two guinea-pigs were also inoculated with negative results.

Mouse.—Eighteen mice were inoculated intraperitoneally, and in addition another twelve intracerebrally, but with negative results.

Man.—Although exposed to infection from cats with feline distemper, no laboratory workers have suffered from illnesses which could be in any way attributed to infection with feline distemper.

Preservation of Virus.

The absence of a supply of kittens bred under conditions eliminating the possibility of chance infections limited the number of experiments in this direction, but definite evidence was obtained that the virus is attenuated or destroyed by ordinary drying but preserves its properties when kept in 50 per cent. glycerine.

Dried Virus.—The spleen and mesenteric lymphatics of a cat killed immediately it showed a rise in temperature, three days after being inoculated with a large dose of virus, were dried *in vacuo* at room temperature in a dessicator containing P_2O_5. The dried substance was kept at room temperature for 19 days, then emulsified in saline, filtered, and the filtrate inoculated into two kittens, Nos. 116 and 117. A third kitten was kept in the adjoining cage as a control. One of the inoculated kittens, No. 117, showed a temperature of 104° F. on the seventh day, but otherwise all three kittens remained normal. Twelve days later Kitten No. 117 was sprayed with infective naso-pharyngeal washings from another cat. Fifteen days later it showed a fever of 105° F. for four days, but blood cultures were sterile. Subsequently it was repeatedly inoculated with virus, but it showed no febrile or other signs of disease.

The other kitten, No. 116, was similarly sprayed with infective material 14 days after the original inoculation, but showed no signs of infection during 19 days, when it was again inoculated with virus. It was found dead six days later, but the cause of death was uncertain, as there was no congestion of the intestine and the only pathological change observed was some congestion in the lungs.

The control kitten remained normal for 23 days and was then exposed to contact with a blanket from a case of the disease. Six days later it showed a rise in temperature, but blood cultures were sterile, and it was killed *in extremis* after 22 days. It showed congestion of the lower part of the small intestine. A strain of *Salmonella* was found in cultures of the blood and various organs.

A second experiment was made, using blood collected from a kitten on the first day of fever, five days after being inoculated with a large dose of virus. After drying at room temperature *in vacuo*, the dried blood was kept for 15 days, then mixed with saline and passed through a Berkefeld filter. Two kittens, Nos. 119 and 120, were inoculated intraperitoneally with this filtrate. Neither of them showed any febrile symptoms or other signs of disease, and respectively 12 and 14 days later were each sprayed with infected nasopharyngeal washings. Eighteen days later one of the kittens, No. 119, had fever for two days, but blood cultures were negative. Both these animals were repeatedly re-inoculated with virus but remained well during the succeeding nine months they were under observation.

Glycerinated Virus.

Suspensions of the spleen and mesenteric lymphatic glands in 50 per cent. glycerine have been found to retain their virulence for varying periods as shown in the following experiments :—

Kitten No. 166, after being isolated for three weeks, was inoculated subcutaneously with a suspension of spleen and mesenteric lymphatic glands kept in 50 per cent. glycerine in the ice chest for 21 days. The suspension was passed through a Berkefeld filter before being used for inoculation. This kitten died with typical symptoms of the disease nine days later.

Kitten No. 236, inoculated intraperitoneally with a culture of a hæmophilic organism isolated from one of the cats dead of distemper, showed no signs of infection during ten days. It was then inoculated intraperitoneally with a Berkefeld filtrate of infected spleen and mesenteric lymph glands which had been kept in 50 per cent. glycerine for 17 days. The kitten was killed *in extremis* seven days later, and cultures of the blood, trachea, lung, and spleen were sterile. The mesenteric gland contained a *Streptococcus*. It is of interest that, although inoculated with a large dose of a hæmophilic organism isolated from a cat dead of distemper, no trace of this organism could be found at the post-mortem examination.

Kittens Nos. 252 and 253 were each inoculated with a Berkefeld filtrate of glycerinated spleen and mesenteric lymph glands kept in the ice chest for 17 days. Both animals became ill and were killed respectively ten and eleven days later. Each showed typical symptoms of feline distemper.

Immunisation.

We have already referred to the results obtained with the inoculation of dried virus. This offers a possible method of protecting kittens against infection with the disease, but since the protection seems to depend on the production of a mild attack by means of attenuated virus its use would be attended by many difficulties. The main objection to this method would be the possibility of the attenuated virus regaining its full virulence, especially after passage. The ideal method of immunisation in feline distemper, as in canine distemper, would seem to be the injection of a dead or attenuated virus followed by live virus or by simultaneous injections of virus and serum. It is hoped that experiments will be carried out on these lines. In the meantime, the injection of immune serum, though the immunity to which it gives rise is probably only temporary, may be of use to veterinarians in the case of valuable cats immediately before their appearance at cat shows.

The following experiments show that a certain degree of immunity may be conferred by immune serum collected from cats which had recovered from distemper, and had since been repeatedly inoculated with infective material.

Experiment 1.—Three kittens were each inoculated subcutaneously with 2 c.c. of immune serum from Cats 117 and 119, followed an hour later by an injection of virus suspension. One of the kittens died seven days later with no very marked pathological changes except anæmia; its blood contained a *Streptococcus*. The remaining two were both killed *in extremis* on the fifteenth day, and showed typical congestion of the small intestine, combined, in one individual, with a well marked tracheitis. The latter was infected with a *Pasteurella*, but cultures of the blood, trachea, lungs, spleen, and mesenteric lymph glands of the other kitten remained sterile. Three control kittens each inoculated with virus alone all died with typical symptoms.

Either the dose of serum in these cases was insufficient, or the serum had a very low titre of antibodies.

Experiment 2.—Nine kittens were each inoculated subcutaneously with 10 c.c. of the pooled serum of a number of immune cats. Nine days later they were each given a second inoculation of 6 c.c. of the same serum. The following day they were each inoculated subcutaneously with a filtered

suspension of virus from three infected cats, and at the same time nine normal cats were inoculated as controls. With two exceptions, these eighteen kittens belonged to four litters, and half of each litter was used for the protection test and the other half as controls, in order to make the test as even as possible. Moreover, they were all kept together in one room in adjoining cages. Out of the nine kittens inoculated with immune serum two died, respectively 14 and 19 days later ; the first of these showed pneumonic symptoms but no congestion of the intestine, whilst the second showed no obvious pathological changes. The remaining seven kittens have remained well up to date, and also shown no febrile reaction, although re-inoculated with a suspension of virus. Out of the nine controls seven died with typical symptoms, one became ill but recovered, and the remaining one showed no signs of infection.

The results of this experiment suggest that by the inoculation of large doses of immune serum kittens may be protected at least for a short time against feline distemper.

Treatment.

Hitherto no efficient treatment of this disease has been devised, and therefore various experiments were made to determine the effect of immune or hyperimmune sera on infected animals. The results have been uniformly negative, and indicate that once the infection has developed the subsequent injection of immune serum has no appreciable influence on the course of the disease.

Discussion of Results.

The susceptibility of cats to infection by means of fomites is a very serious obstacle to the study of this disease, as elaborate precautions are necessary in order to eliminate the possibility of chance infections. The extent to which infection spreads in the absence of direct contact is, however, open to discussion, as occasionally we have kept normal kittens in the same room as infected ones without any signs of infection or immunisation developing, for when subsequently inoculated with sterile filtrate from cases of the disease they have succumbed. When making any critical experiments we have tried to minimise the risk of chance infections by keeping the kittens under observation for three or four weeks in a separate room before using them for any purpose. Attendants feeding these animals, &c., were required to change their overalls and wash their hands in lysol before entering the room. The greatest difficulty has always been the supply of " clean " kittens. The shops of animal dealers are notoriously infected with this disease, and kittens that had been kept in such quarters were frequently infected before arrival. The only method of reducing this risk was to obtain kittens direct from private houses, and in such cases the animals were found to remain free from infection if reasonable precautions were taken after their arrival. Such kittens were used in experiments with fomites and on the preservation of virus, &c. Unfortunately this supply was very limited and consequently the number of our experiments has been restricted.

The disease itself presents similar difficulties to those encountered in the study of other virus infections which, as a rule, do not directly cause the death of their host. Human influenza and canine distemper would seem to be very closely allied infections and in both an ætiological significance has been attached to a variety of organisms. There is now conclusive evidence that the canine disease is due to a filterable virus (Laidlaw and Dunkin, 1926), and there is some evidence to suggest that the primary agent of human influenza may also be a filterable virus.

In the case of feline distemper the pathological changes produced by the virus, notably the congestion of the small intestine, are those which would favour the invasion of organisms from the alimentary tract, and the results of bacteriological examinations support this expectation. The abdominal lymph glands were the most commonly infected, and in descending order the spleen, lungs, and heart-blood, whilst in 8 per cent. of the total all these tissues were sterile. Moreover, the large variety of bacteria isolated also supports the view that none of them was ætiologically related to the disease.

In experiments on the absorption of *Bacillus prodigiosus* through the wall of the duodenum of dogs, Boone, Chase and Brink (1931) have recently found that, though slight mechanical abrasions of the mucosa did not cause any increase in the number of organisms absorbed into the portal vein, nevertheless a definite increase in normal duodenal absorption was noted as a result of local passive congestion produced by mechanical obstruction of the portal vein.

It is unfortunate that we were unable to discover any certain method of diagnosis, such as characteristic blood changes, or inclusion bodies, for even during epidemics some of the cats, especially young kittens, died without showing any characteristic clinical symptoms. Blood counts were made in a certain number of cats and the earlier results suggested the presence of a monocytosis, but later results were so conflicting that no reliance could be placed on this as a method of diagnosis. A careful search has also been made for the presence of any characteristic cytological appearances in the nucleus or cytoplasm of the cells of infected animals, but hitherto without success.

The results of our protection experiments suggest that kittens may be immunised, at least temporarily, against feline distemper by the use of large doses of immune serum. The difficulties in the use of this method for the immunisation of kittens would seem to be in the large doses of immune serum necessary to give efficient protection. In our experiments, however, very large doses of virus were subsequently inoculated, and it is probable that smaller doses of serum, if given previously, might protect a kitten against chance infections at cat shows. Further experiments, however, are necessary, preferably with kittens bred under conditions eliminating the possibility of chance infection, in order to determine the best methods of protection.

Conclusions.

1.—The clinical symptoms of feline distemper are very variable and offer no certain method of clinical diagnosis.

2.—Pathologically there is constantly present some degree of congestion of the small intestine.

3.—Enlargement and congestion of the abdominal lymph glands is generally observed, and, in addition, enlargement of the spleen, pleurisy, and peritonitis may also occur.

4.—A variety of bacteria have been isolated—*Salmonella*, coliform organisms, *Streptococci*, *Pasteurella*, parainfluenza bacilli, but 8 per cent. of the cases have been bacteriologically sterile.

5.—There is no evidence that any of the above bacteria play an ætiological rôle in feline distemper.

6.—Feline distemper can be transmitted by fomites.

7.—The disease can be transmitted by Berkefeld filtrates of the nasal washings of cats with distemper, or by filtrates of the spleen and abdominal lymph glands.

8.—The disease can be communicated to cats and carried on in series by intranasal instillation of such filtrates and by subcutaneous, intraperitoneal, and intracerebral inoculation.

9.—The primary cause of cat distemper is a filterable virus.

10.—In addition to the domestic cat, the leopard, *Felis pardus*, American Tiger-Cat (*F. tigrina*) ; African Tiger Cat, (*F. aurata*), the Rusty Tiger Cat (*F. planiceps*), the Marbled Cat (*F. marmorata*), the Caracal Lynx (*F. caracal*) and Ocelot (*F. pardalis*) are susceptible.

11.—Dogs, ferrets, mongooses, rabbits, guinea-pigs, and mice are insusceptible.

12.—The virus may be preserved in 50 per cent. glycerine.

13.—The virus is attenuated or killed by drying at room temperature. The inoculation of such dried virus is followed by some degree of immunisation.

14.—The injection of large doses of immune serum protects kittens, at least temporarily, against inoculations of virus.

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THE PRESENCE OF AGGLUTININS FOR BACTERIA OF THE SALMONELLA GROUP IN THE SERA OF NORMAL ANIMALS.

A PRELIMINARY REPORT.*

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The following communication is concerned with the agglutinins for the " H " heat labile flagellar antigens of certain members of the Salmonella group, which occur in the sera of healthy domesticated animals. Special attention has been paid to swine, in view of the widespread opinion that *Bact. suipestifer* is a normal inhabitant of healthy swine.

Technique Employed.

Bacterial suspensions were prepared by growing the organisms in broth for 16 hours at 22° C. and then killing by the addition of formalin to a concentration of 0·25 per cent. In the case of the diphasic members of the group it was necessary first to select colonies of the required phase from plate cultures. Cultivation at the low temperature of 22° C. for the period of 16 hours enabled one to procure suspensions of sufficient opacity and yet mutation of phase rarely occurred. All suspensions were rigorously tested with homologous and heterologous sera before use. The affinity between *Bact. enteritidis* and *Bact. derby* and also between the specific phases of Newport and Reading were noted. These have previously been recorded by Andrewes (1925) and White (1925 and 1926). All suspensions were standardised to contain approximately 500 million organisms per c.c. and sufficient suspensions were prepared for the whole of the investigation. The following is a list of the strains of bacteria used :—

(1) *Bact. paratyphosum* B, strain " Grey " of the Public Health Laboratory, Manchester.

(2) *Bact. ærtrycke*, N.C.T.C. 74, isolated from food poisoning in man, Newcastle, 1911.

(3) *Bact. newport*, N.C.T.C. 129, isolated from fatal case of food poisoning.

(4) *Bact. reading*, N.C.T.C. 72, isolated by Schütze from water supply at Reading in 1916.

(5) *Bact. derby*, N.C.T.C. 1729, isolated by Peckham (1923) from pork-pies at Derby.

* The data recorded in this paper were incorporated in a thesis submitted for the Degree of M.Sc., in the University of Manchester.

(6) *Bact. suipestifer*, N.C.T.C. 179, " Arkansas." This strain is stated by Schütze (1921) to be a rough variant of the Hog Cholera type, but by using the technique previously described smooth homogeneous suspensions were easily obtained. The strain is also purely specific in its phase characteristics (White, 1926).

(7) *Bact. enteritidis*, N.C.T.C. 126, isolated by Hutchens from milk that produced food poisoning at Newcastle 1910. This strain of *Bact. enteritidis* is in reality one of the Dublin type of Salmonella (Smith and Scott, 1930). It will, however, be referred to throughout this communication as *Bact. enteritidis*. (The flagellar antigens of the two organisms are very similar, the formula for *Bact. enteritidis* being $R.P_1P_2$ whilst the Dublin type is represented as $R.P_1P_3$.)

Collection of Samples of Sera.

The blood was collected from various slaughter-houses in Manchester and London. The particulars of each animal, with special reference to approximate age, sex, breed, state of health at time of death, and whether any abnormalities were found in the carcase, were received with each sample. All animals from which blood was obtained were as far as could be ascertained in a perfect state of health before slaughter and the carcase passed as fit for the food of man. The samples of horse sera were from normal horses kept at the Wellcome Physiological Research Laboratories.

Agglutination Technique.

The agglutination experiments were performed in accordance with Dreyer's technique, the exception being that graduated pipettes were used for making the dilutions and the results recorded in dilutions and not in units. The dilutions used were as follows : 1/20, 1/40, 1/80, 1/160, 1/320, 1/640 and 1/1,280. The tubes were incubated in a water bath at 55° C., and readings were taken at the end of two hours and again after 24 hours. There was usually no appreciable difference between the two readings.

Absorption Technique.

A number of sera which agglutinated several of the organisms were re-tested by absorption. The following method was employed : Into a centrifuge tube were delivered :—

1 c.c. of undiluted serum.
2 c.c. of normal physiological saline.
2 c.c. of thick saline suspension of the absorbing organism.

A saline control was included with each serum. The tubes were shaken at frequent intervals during the day and left in the ice chest overnight. The following morning they were centrifugalised and the clear serum removed, test agglutinations being carried out as previously described. It will be observed that the absorption took place at a dilution of 1/5, and it was thus possible to carry out the test agglutinations by starting at a dilution of 1/20.

Results of Experiments.

Titres are expressed in dilutions and the sign " O " denotes that no agglutination was detectable at a dilution of 1/20. In certain tables, the following abbreviations are used :—

B(g)	denotes	*Bact. paratyphosum* B	(group phase)
B(t)	„	*Bact. paratyphosum* B	(type phase)
D	„	*Bact. derby*	
A	„	*Bact. ærtrycke*	(type phase)
N	„	*Bact. newport*	(type phase)
R	„	*Bact. reading*	(type phase)
S	„	*Bact. suipestifer*	(type phase)
E.G.	„	*Bact. enteritidis.*	

Swine.

TABLE I (202 Swine.)

The following table shows the number of sera which agglutinated the various strains at a dilution of 1/20 or over, and their corresponding percentages.

Bact. paratyphosum B	(group phase)	by	129	=	63·9%
„ *paratyphosum* B	(type phase)	„	83	=	41·1%
„ *derby*		„	52	=	25·7%
„ *ærtrycke*	(type phase)	„	94	=	46·5%
„ *newport*	(type phase)	„	127	=	62·9%
„ *reading*	(type phase)	„	24	=	11·9%
„ *suipestifer*	(type phase)	„	121	=	59·9%
„ *enteritidis*		„	86	=	42·6%

The results of the agglutination experiments showed several points of interest. The titres were comparatively low, ranging from 1/20 to 1/320. It is shown in Table I that the suspensions most commonly agglutinated were :—

Bact. paratyphosum B	(group phase)
„ *newport*	(type phase)
„ *suipestifer*	(type phase)
„ *ærtrycke*	(type phase)

Bact. paratyphosum B (type phase) and *Bact. enteritidis* were also agglutinated by over 40 per cent. of the sera. The rare occasions on which *Bact. reading* (type phase) was affected is of interest in view of the antigenic relationship which has been pointed out between the specific phases of Reading and Newport. The sera examined were random samples and, as previously stated, were not collected from one slaughter-house but from several. The breeds of the pigs also varied and included such as Large Black, Essex, Gloucester Old Spot, Yorkshire, and many of a mixed variety. An analysis of the titres in respect to the various suspensions is shown in Table II.

It is indicated in Table II that the commonest titre is 1/20, and in the majority of cases there is a gradual decline in frequency as higher titres are approached. *Bact. suipestifer* is the only organism which was agglutinated in a dilution of 1/640.

TABLE II (202 SWINE.)

An analysis of the titres of the sera of 202 swine.

	B(g).	*B(t).*	*D.*	*A.*	*N.*	*R.*	*S.*	*E.G.*
—	73 = 36·1%	119 = 58·9%	150 = 74·2%	108 = 53·5%	75 = 37·3%	178 = 88·1%	81 = 40·1%	116 = 57·9%
1/20	42 = 20·8%	39 = 19·3%	35 = 17·3%	35 = 17·3%	60 = 29·7%	15 = 7·4%	45 = 22·3%	51 = 25·2%
1/40	43 = 21·3%	28 = 13·9%	10 = 5·0%	32 = 15·8%	32 = 15·8%	4 = 2·0%	28 = 13·9%	18 = 8·9%
1/80	30 = 14·8%	13 = 6·4%	6 = 3·0%	17 = 8·4%	22 = 10·9%	4 = 2·0%	28 = 13·9%	13 = 6·4%
1/160	10 = 4·0%	2 = 1·0%	0	9 = 4·4%	10 = 4·9%	1 = 0·5%	14 = 6·9%	2 = 1·0%
1/320	4 = 1·2%	1 = 0·5%	1 = 0·5%	1 = 0·5%	3 = 1·5%	0	4 = 2·0%	2 = 1·0%
1/640	0	0	0	0	0	0	2 = 1·0%	0
1/1280	0	0	0	0	0	0	0	0

TABLE III (202 Swine).

	(32 *Swine*) 5 *months and under.*	(124 *Swine*) 5-8 *months.*	(38 *Swine*) 9 *months and over.*
B(g)	20 = 62·5%	68 = 54·8%	34 = 89·5%
B(t)	15 = 46·9%	43 = 34·7%	21 = 55·3%
D	9 = 28·1%	26 = 21·0%	15 = 39·5%
A	16 = 50·0%	51 = 41·1%	24 = 63·1%
N	25 = 78·1%	66 = 53·2%	30 = 76·3%
R	7 = 21·9%	13 = 10·5%	4 = 10·5%
S	22 = 68·7%	68 = 54·8%	26 = 68·4%
E.G.	15 = 46·9%	46 = 37·0%	22 = 57·9%

The ages of eight swine were unknown.

Age.

As shown in Table III, an attempt has been made to ascertain whether there was any correlation between the age of the animal and the presence of normal agglutinins. The difficulty encountered in dealing with swine is that they attain puberty at about five months, and few swine are killed at an earlier age. Further, the age of the pigs is merely approximate, because of the difficulty of ascertaining the age of a pig with any degree of accuracy. The table indicates that the swine examined have been divided into three groups :—

(*a*) Five months old and under.
(*b*) Five to eight months old.
(*c*) Nine months and over.

There is no outstanding evidence obtainable from this table of any correlation. There is, however, a tendency for the swine over nine months old to show a more marked reaction than the younger ones. The majority of the higher titres (1/320) were met with in the older animals ; the sera of these older animals also tended to agglutinate a greater range of antigens than the younger ones. The sera of the two youngest dealt with (age two and a half months) each agglutinated five suspensions, but in no case was a higher titre than 1/20 recorded. It will be noted also that the frequencies for the five to eight months group are in almost every case lower than those for the under five months or nine months and over group. The differences except in a few instances are not great, and it is impossible on this series of tests alone to judge of their significance.

TABLE IV (202 Swine).

	Female (98).	*Male* (91)
B(g)	66 = 67·3%	52 = 57·1%
B(t)	43 = 43·9%	35 = 38·9%
D	26 = 26·5%	21 = 23·1%
A	50 = 51·0%	37 = 40·6%
N	66 = 67·3%	52 = 57·1%
R	11 = 11·2%	12 = 13·2%
S	59 = 60·2%	53 = 58·2%
E.G.	46 = 46·4%	35 = 38·4%

The sex of 13 swine was not known.

The 202 swine were divided into two groups, male and female, and Table IV indicates the findings. It would appear that the sera of female swine are more liable to contain agglutinins than those of male swine ; the figures are barely sufficient to indicate whether this difference is significant.

Discussion of Swine Results.

Of the 202 swine examined, the sera of 190 agglutinated one or more organisms to a titre of 1/20 or above. The actual titre was obtained in each case, dilutions being carried to 1/1,280, and no serum agglutinated any organism beyond a dilution of 1/640. The summary of the results is indicated in Tables I and II. The strains most commonly agglutinated are recorded there. The highest titre reached has been in the case of *Bact. suipestifer* on two occasions, when 1/640 was recorded. The usual titres were from 1/20 to 1/80. The difficulties met with in attempting to correlate the age of the pig and the presence of normal agglutinins has been explained and, although Table III gives little information, there appears to be a tendency for agglutinins to increase with age. Table IV indicates that the sera of females are more liable to contain agglutinins than those of male swine.

A list is appended giving the number of sera which agglutinated varying numbers of suspensions.

12	sera	(5·9%)	did not agglutinate any suspension.
30	„	(14·8%)	agglutinated one suspension.
31	„	(15·3%)	„ two suspensions.
30	„	(14·8%)	„ three „
28	„	(13·8%)	„ four „
28	„	(13·8%)	„ five „
24	„	(12·4%)	„ six „
12	„	(5·9%)	„ seven „
6	„	(3·0%)	„ eight „

This shows clearly that the majority of sera examined agglutinated more than one species of organism.

Cattle.

TABLE V (23 Cattle).

The following Table shows the number of sera which agglutinated the various organisms at a dilution of 1/20 or over and their corresponding percentages.

Bact. paratyphosum B	(group phase)	by	4	=	17·4%
„ *paratyphosum* B	(type phase)	„	4	=	17·4%
„ *derby*		„	7	=	30·4%
„ *ærtrycke*	(type phase)	„	11	=	47·8%
„ *newport*	(type phase)	„	11	=	47·8%
„ *reading*	(type phase)	„	2	=	8·7%
„ *suipestifer*	(type phase)	„	10	=	43·5%
„ *enteritidis*		„	10	=	43·5%

Of the 23 cattle sera examined, 13 produced some reaction, the titres ranging between 1/20 and 1/80 (except in the case of two which exceeded 1/80). Although comparatively few sera have been examined, the organisms most frequently agglutinated were :—

Bact. ærtrycke (type phase)
Bact. newport (type phase)
Bact. suipestifer (type phase)
Bact. enteritidis.

TABLE VI.

	Under 1 *year old* (11 *cattle*).	*Over* 1 *year old* (12 *cattle*).
B(g)	0	4 = 33·3%
B(t)	1 = 8·3%	3 = 25·0%
D	0	7 = 58·3%
A	0	11 = 91·6%
N	0	11 = 91·6%
R	0	2 = 16·6%
S	0	10 = 83·3%
E.G.	0	10 = 83·3%

Age.

The difference in the results, according to the age of the animal, is well shown in Table VI. Of the 23 examined, only one of the young animals (eleven in number) contained or showed evidence of the presence of agglutinins to the bacteria tested, and that only in a dilution of 1/20. There is evidence in this small number that normal agglutinins for bacteria of the Salmonella group are rare in young bovines. The division into the two age groups is based on the assumption that cattle reach puberty at twelve to 18 months of age.

Sheep.

TABLE VII (46 Sheep.)

The following Table shows the number of sera which agglutinated the various organisms at a dilution of 1/20 or over and their corresponding percentages.

Bact. paratyphosum B	(group phase)	by	3 =	6·5%
„ *paratyphosum* B	(type phase)	„	0 =	0
„ *derby*		„	3 =	6·5%
„ *ærtrycke*	(type phase)	„	17 =	36·9%
„ *newport*	(type phase)	„	6 =	13·0%
„ *reading*	(type phase)	„	0 =	0
„ *suipestifer*	(type phase)	„	30 =	65·2%
„ *enteritidis*		„	3 =	6·5%

Of the 46 sheep examined, 33 reacted to one or more of the suspensions. Reference to the above table will indicate that the agglutinins are less numerous ; they are also less developed, except in the case of *Bact. suipestifer*, than in the other animals dealt with. *Bact. suipestifer* reacted positively to 65·2 per cent. and *Bact. ærtrycke* to 36·9 per cent. of the sera ; otherwise the reactions are poorly developed and the titres comparatively low.

TABLE VIII (46 Sheep.)

	Under six months old. (14 *sheep.*)	*Over six months old.* (29 *sheep.*)
B(g)	0	3 = 10·3%
B(t)	0	0
D	0	3 = 10·3%
A	0	15 = 51·7%
N	0	6 = 20·7%
R	0	0
S	3 = 21·42%	24 = 82·7%
E.G.	0	2 = 6·9%

The ages of three sheep were unknown.

Age.

Table VIII is illuminating as showing the comparative absence of agglutinins in young sheep. The number examined has been divided into two sections :—

(*a*) Sheep under six months of age.

(*b*) Sheep six months old and over.

As indicated in the table, only three of the young sheep showed any reaction. The sera in these cases agglutinated *Bact. suipestifer* two to a titre of 1/20 and one to a titre of 1/40. There is clear evidence, on the number examined, that normal agglutinins for bacteria of the Salmonella group are absent or poorly developed in young sheep.

Horses.

TABLE IX (20 Horses.)

The following Table shows the number of sera which agglutinated the various organisms at a dilution of 1/20 or over and their corresponding percentages.

Bact.	*paratyphosum* B	(group phase)	by	7	= 35%
,,	*paratyphosum* B	(type phase)	,,	3	= 15%
,,	*derby*		,,	3	= 15%
,,	*ærtrycke*	(type phase)	,,	7	= 35%
,,	*newport*	(type phase	,,	9	= 45%
,,	*reading*	(type phase)	,,	11	= 55%
,,	*suipestifer*	(type phase)	,,	8	= 40%
,,	*enteritidis*		,,	7	= 35%

Of the 20 horses examined, 18 showed moderately developed agglutinins ; with one exception, the titres are not higher than 1/160. The number is small and, as previously pointed out, the sample as a whole suffers from the disadvantage that nearly all the horses are living in the same area and under identical conditions. The ages of only a few were available and, as they were all over nine years of age, no conclusions can be drawn regarding the correlation of age and the presence of normal agglutinins. The fact that *Bact. reading* was agglutinated by 55 per cent. of the sera is worthy of note, when one compares the small percentage of sera of other animals showing any agglutination with that organism.

Fractional Absorption of Sera.

The following Tables, X, XI and XII, give the results obtained by absorption of some of the sera with various micro-organisms of the Salmonella group. The technique employed has already been described.

TABLE X (Swine).

Absorption of Sera with Various Organisms.

No.	Sera.	B(g).	B(t).	D.	A.	N.	R.	S.	E.G.
1	unabsorbed control	80	40	160	80	80	0	80	160
	absorbed with B.	0	0	160	80	80	0	80	160
	„ „ A.	20	40	160	0	0	0	80	160
	„ „ N.	20	20	160	80	0	0	80	160
4	unabsorbed control	20	0	0	160	20	0	20	20
	absorbed with A.	0	0	0	0	20	0	0	20
	„ „ N.	0	0	0	160	0	0	20	20
16	unabsorbed control	20	0	0	40	160	0	20	0
	absorbed with N.	0	0	0	40	0	0	20	0
33	unabsorbed control	0	160	0	20	0	0	20	0
	absorbed with B.	0	0	0	20	0	0	20	0
47	unabsorbed control	20	80	0	20	320	0	40	0
	absorbed with N.	0	80	0	20	0	0	40	0
49	unabsorbed control	20	80	0	160	40	0	80	0
	absorbed with A.	0	80	0	0	20	0	80	0
56	unabsorbed control	40	20	80	0	80	0	40	80
	absorbed with N.	0	20	80	0	0	0	40	80
	„ „ S.	0	20	80	0	40	0	0	80
57	unabsorbed control	20	20	80	80	320	0	80	80
	absorbed with N.	0	20	80	80	20	0	40	80
	„ „ S.	0	20	80	40	160	0	0	80
62	unabsorbed control	40	40	0	0	80	0	40	0
	absorbed with B.	0	0	0	0	80	0	40	0
	„ „ N.	0	40	0	0	0	0	20	0
63	unabsorbed control	40	40	0	80	0	0	40	0
	absorbed with B.	20	0	0	80	0	0	80	0
	„ „ A.	20	40	0	0	0	0	20	0
	„ „ S.	20	40	0	20	0	0	0	0
68	unabsorbed control	40	80	0	0	0	0	40	20
	absorbed with B.	0	0	0	0	0	0	40	20
	„ „ S.	0	80	0	0	0	0	0	20
78	unabsorbed control	160	0	0	20	20	0	40	20
	absorbed with N.	20	0	0	0	0	0	40	20
	„ „ S.	160	0	0	20	20	0	0	20
79	unabsorbed control	20	80	20	80	40	0	40	20
	absorbed with B.	0	0	20	80	20	0	40	20
	„ „ A.	0	80	20	0	20	0	40	20
80	unabsorbed control	80	40	0	20	40	0	40	40
	absorbed with B.	40	0	0	20	40	0	40	40
	„ „ N.	0	40	0	20	0	0	40	40
	„ „ S.	20	40	0	20	40	0	0	40
84	unabsorbed control	0	0	0	40	0	0	80	0
	absorbed with S.	0	0	0	40	0	0	0	0

TABLE X (*continued*).

No.	*Sera.*	*B(g).*	*B(t).*	*D.*	*A.*	*N.*	*R.*	*S.*	*E.G.*
87	unabsorbed control	20	0	0	0	0	0	80	0
	absorbed with S.	0	0	0	0	0	0	0	0
91	unabsorbed control	160	40	40	160	40	20	20	40
	absorbed with B.	40	0	20	160	20	0	0	40
	„ „ A.	20	40	40	0	40	0	0	40
94	unabsorbed control	40	0	0	0	20	0	40	0
	absorbed with N.	0	0	0	0	0	0	20	0
	„ „ S.	0	0	0	0	20	0	0	0
95	unabsorbed control	80	40	0	20	40	0	80	0
	absorbed with B.	40	0	0	20	40	0	80	0
	„ „ N.	0	20	0	0	0	0	80	0
	„ „ S.	40	20	0	20	40	0	0	0
96	unabsorbed control	0	0	0	0	20	0	40	0
	absorbed with N.	0	0	0	0	0	0	20	0
	„ „ S.	0	0	0	0	20	0	0	0
97	unabsorbed control	40	0	0	0	40	0	40	0
	absorbed with N.	0	0	0	0	0	0	20	0
	„ „ S.	20	0	0	0	20	0	0	0
98	unabsorbed control	80	40	0	0	40	0	80	0
	absorbed with B.	40	0	0	0	20	0	80	0
	„ „ S.	20	20	0	0	20	0	0	0

TABLE XI (CATTLE.)

No.	*Sera.*	*B(g).*	*B(t).*	*D.*	*A.*	*N.*	*R.*	*S.*	*E.G.*
5	unabsorbed control	0	0	0	0	20	0	20	20
	absorbed with N.	0	0	0	0	0	0	20	20
7	unabsorbed control	20	0	40	20	40	0	0	40
	absorbed with A.	0	0	40	0	40	0	0	40
	„ „ N.	0	0	40	20	0	0	0	40
10	unabsorbed control	20	0	20	160	40	0	20	20
	absorbed with A.	0	0	0	0	0	0	0	20
	„ „ S.	0	0	20	160	40	0	0	20
12	unabsorbed control	0	0	40	80	20	0	0	20
	absorbed with A.	0	0	20	0	0	0	0	20
	„ „ N.	0	0	25	40	0	0	0	0

TABLE. XII (Sheep).

No.	*Sera.*	*B(g).*	*B(t).*	*D.*	*A.*	*N.*	*R.*	*S.*	*E.G.*
3	unabsorbed control	0	0	0	160	0	0	160	0
	absorbed with A.	0	0	0	0	0	0	0	0
7	unabsorbed control	0	0	0	40	0	0	20	0
	absorbed with A.	0	0	0	0	0	0	0	0
	„ „ S.	0	0	0	0	0	0	0	0
8	unabsorbed control	20	0	0	0	0	0	20	0
	absorbed with A.	0	0	0	0	0	0	0	0
	„ „ S.	0	0	0	0	0	0	0	0
9	unabsorbed control	0	0	40	40	0	0	40	0
	absorbed with A.	0	0	20	0	0	0	0	0
	„ „ S.	0	0	20	20	0	0	0	0
12	unabsorbed control	0	0	20	20	0	0	40	0
	absorbed with A.	0	0	20	0	0	0	0	0
	„ „ S.	0	0	20	20	0	0	0	0
13	unabsorbed control	0	0	0	80	0	0	320	0
	absorbed with A.	0	0	0	0	0	0	160	0
	„ „ S.	0	0	0	0	0	0	0	0
16	unabsorbed control	0	0	0	0	80	0	160	0
	absorbed with N.	0	0	0	0	0	0	160	0
	„ „ S.	0	0	0	0	0	0	0	0
21	unabsorbed control	20	0	0	0	80	0	40	0
	absorbed with N.	0	0	0	0	0	0	20	0
	„ „ S.	0	0	0	0	0	0	0	0

The absorption results show that in the majority of cases the agglutinins can be removed by the homologous organism and the others left intact. Swine No. 1, Table X, is quite a good example, for in this case *Bact. paratyphosum* B removed the agglutinins for its group and type, but did not affect those for *Bact. derby*, *ærtrycke*, *newport*, *reading*, *suipestifer*, or *enteritidis*. Similar results were obtained with the absorption by *Bact. ærtrycke* and *newport*. There is, however, a reduction in the titre of *Bact. paratyphosum* B (group phase), which was to be expected. It must be pointed out that there is often a relationship between *Bact. ærtrycke* and *Bact. suipestifer* which cannot be explained. For example, absorption with *Bact. ærtrycke* has removed not only its own homologous agglutinins but in addition the agglutinins for *Bact. suipestifer*, in the sera of Swine Nos. 4 and 91, Cattle No. 10, Sheep Nos. 3, 8, 9 and 12. *Bact. suipestifer* has in the case of Swine No. 66, and Sheep 7 and 13, removed the agglutinins for *Bact. ærtrycke*, and the agglutinins for *Bact. newport* in Sheep Nos. 16 and 21. The results are not clear-cut, but one can at least say that the homologous agglutinins can be absorbed, and the heterologous ones left intact in most cases. The agglutinins therefore appear to be specific in the majority of cases ; for some unknown

reason, they do not appear to be so in all. . Specificity is not well marked in sheep, for it is only in the minority of cases that the results are in accordance with what one might expect.

General Discussion of Results.

There are considerable differences between the results of the examination of the sera of the various animals. Swine are the only animals whose sera agglutinated *Bact. paratyphosum* B to any extent, and the group phase is the one most frequently affected. Next in order are the type phases of *Bact newport* and *Bact. suipestifer*. In the case of cattle *Bact. ærtrycke*, *Bact. newport*, *Bact. suipestifer*, and *Bact. enteritidis* are those most frequently agglutinated, with *Bact. derby* next in order. The results of the agglutinations by the sera of sheep show a great difference ; the agglutinins are much more scarce and in the majority of cases poorly developed. There is an exception in the case of *Bact. suipestifer*, which was agglutinated by 65 per cent. of the sera and in two cases to a titre of 1/640. The only other suspension which was agglutinated to any extent was *Bact. ærtrycke*. The specific phases of *Bact. paratyphosum* B, and *Bact. reading* were not agglutinated by any of the sheep sera tested. It is hardly permissible to compare the results of the horse sera with the others, as the majority of the horses are living under similar conditions in the same area. Notwithstanding this, one feels justified in pointing out that *Bact. reading* was agglutinated by 55 per cent. of the sera examined. This is very different from the other results, for it was not agglutinated by any of the sheep sera, and only by 11·8 per cent of swine and 8·7 per cent. of cattle. There is some evidence in the case of cattle and sheep that normal agglutinins are absent in the young animal. This has already been pointed out under the respective headings, and also the difficulties of correlating age with the presence or absence of normal agglutinins in pigs.

Attempted Cultivation of Organisms of the Salmonella Group from Swine.

It was thought that a little light might be shed upon the significance of these agglutinins if an attempt to cultivate organisms of the Salmonella group from the organs or fæces of healthy swine was made. Accordingly visits were made to various slaughter-houses and cultivation attempted. The technique employed was as follows :—

About 20 c.c. of blood was caught as the animal was killed, and the serum used for agglutination tests.

The surface of the gall bladder was seared with a hot iron and 5 c.c. of bile withdrawn into a sterile syringe and discharged into a sterile test tube. On return to the Laboratory this was transferred to 100 c.c. of casein broth. The spleen and liver were seared over a small area and a portion of each organ removed with sterile scissors and forceps, and dropped into 10 c.c. of casein broth. A similar technique was employed when dealing with the pharyngeal lymphatic

glands. All cultures were incubated at 37° C., and when there was any evidence of growth plate cultures were prepared on to McConkey's medium and sub-cultures into casein broth to which Brilliant Green had been added. No tube or flask which appeared to remain sterile was discarded until it had been incubated for at least five days at 37° C. and then plated on to McConkey's agar medium. All plates were examined for non-lactose fermenting colonies ; if these were found films were prepared and the organism investigated further.

The following is a list of the cultures made from normal healthy swine :—

From the intestinal contents	18	samples.
„ liver	38	„
„ bile	26	„
„ spleen	28	„
„ pharyngeal lymphatic gland ..	12	„
Total	132	

In all 132 samples were examined from 40 different pigs, all of which showed the presence of agglutinins for the Salmonella group in their sera. Many lactose-fermenting organisms and occasionally cocci were isolated from the various tissues, but in no case was any organism of the Salmonella group isolated. As no organism of that nature could be found, it was thought advisable to study some of the organisms isolated, with a view to establishing the presence or otherwise of any antigenic relationship between them and members of the group. Accordingly such bacteria, which had been isolated particularly from the spleen or pharyngeal lymphatic glands of the pigs, were first identified as far as possible by their carbohydrate fermentation reactions, and suspensions were then prepared. In no case were any of them agglutinated by sera which had been prepared against members of the Salmonella group. They were agglutinated in almost every case by the serum of the pig from which they were isolated, usually in dilutions ranging from 1/40 to 1/320 ; but they did not absorb the agglutinins for members of the Salmonella group from the pig sera. Attention was therefore paid to the growth of bacteria from the intestinal contents of the pigs. Twelve samples from swine were grown in 100 c.c. of casein broth at 37° C. for 18 hours and the growth was then filtered through one thickness of filter paper. The turbid filtrate was used as the antigen and agglutination experiments put up against high titre sera prepared against various members of the Salmonella group. No sign of agglutination appeared in any case. The growth from the intestinal contents was also seeded on to agar plates and 150 colonies picked off in duplicate. One series was kept and the other formolised and similar agglutination experiments performed, but in no case could one find any association between Salmonella agglutinins and these cultures.

A further technique was employed in dealing with four samples of intestinal contents. Topley and Fielden (1922) studied broth cultures of human fæces and found that by frequent sub-cultivation on to plates, at definite intervals of time, the dominant species developed in a more or less orderly manner, one species replacing another and in its turn being replaced by a third. By taking advantage of this technique it is possible to isolate particular organisms from a fluid culture in which a highly complex flora is developing. Ten sub-cultures were therefore, made with the four samples over a period of ten weeks, and, although many colonies were studied, and the change in the dominant species was very noticeable, no organism of the Salmonella group was isolated.

Discussion.

As this is a preliminary study it is not proposed to discuss fully the significance of the presence of agglutinins in the sera of normal animals. It must, however, be pointed out that the presence of agglutinins for various bacteria in the sera of healthy animals is not a new observation. It has been noted by Bürgi (1907),Uhlenhuth, Hübener, Xylander and Bohtz (1909), Wehrbein (1916), Savage (1918), Kinloch, Smith and Taylor (1926), Gibson (1930), Timmermann (1930), and by others.

Various hypotheses regarding the significance of these agglutinins have been brought forward :—

(1) That they are due to an old infection with some member or members of the group of bacteria concerned.

(2) That they are due to infection with some other but unrecognised organism which is sufficiently allied to the Salmonella group to cause the serum to have some action on strains belonging to the group.

(3) That they are natural agglutinins arising in the course of normal development and independently of any specific stimulus.

The reports of attempted isolation of bacteria of the Salmonella group from the tissues or intestinal contents of normal healthy animals are also conflicting. Negative findings have been reported by Savage (1906-7), Aumann (1911), Bainbridge (1911), Horn and Huber (1912), Fischer (1915), Jordan (1918), Kinloch, Smith and Taylor (1926). On the other hand there are reports of positive findings in a small percentage of cases by various Continental workers, such as : Uhlenhuth, Hübener, Xylander and Bohtz (1908), Seiffert (1909), Schmidt (1911), Trawínski (1917), Gheorghiu and Costin (1917) and Bermann (1927). The balance of evidence is against such bacteria being normal inhabitants of healthy domesticated animals in this country or America.

A full discussion of the presence and significance of normal agglutinins in healthy domesticated animals will, it is hoped, be published at some future date. Further details of such agglutinins

not only for the " H " heat labile flagellar antigens but for the " O " heat stable somatic antigens will also be recorded.

For the moment it may be noted that these antibodies appear to be specific as judged by absorption tests, and that in cattle and sheep, and to a less extent in pigs, there is clear evidence of their increased frequency with advancing age. No evidence has, however yet been obtained to support the view that they arise as the result of actual infection.

Summary.

1.—The sera of 202 normal healthy swine
23 „ „ cattle
46 „ „ sheep
20 „ „ horses
have been tested for agglutinins against the following " H " heat labile antigens of the Salmonella group :—

Bact. paratyphosum B (group phase)
„ *paratyphosum* B (type phase)
„ *derby*
„ *ærtrycke* (type phase)
„ *newport* (type phase)
„ *reading* (type phase)
„ *suipestifer* (type phase)
„ *enteritidis*

The reactions are given in the foregoing tables, which indicate comparisons between the various animals. Normal agglutinins against one or more of the various strains used are developed in 190 swine, 13 cattle, 33 sheep, and 18 horses.

2.—Normal agglutinins are absent or poorly developed in young sheep and young bovines. All pigs tested (except two) had reached puberty, and all the horses were aged, and so no conclusions regarding age can be given from their results.

3.—There is a tendency for the sera of female swine to possess a higher agglutinin content than the sera of male swine.

4.—Absorptions of sera with various members of the Salmonella group show specificity of agglutinins in most cases, but this specificity is not so well marked in sheep.

5.—Cultivation of members of the Salmonella group from the tissues and intestines of normal swine has been attempted, and there is no evidence to show that they are normal inhabitants of healthy swine.

6.—The agglutination of members of the Salmonella group of bacteria by the sera of swine, cattle, sheep and horses is not in itself evidence of infection with members of the group.

7.—It is hoped to publish later a discussion of the significance of normal agglutinins, and details of further investigations regarding the presence of agglutinins for both " H " and " O " antigens of members of the Salmonella group in the sera of normal domesticated animals.

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STUDIES ON BOVINE MASTITIS.

VI.—The Non-Hæmolytic Streptococci of Bovine Mastitis and their Relationship to Certain Saprophytic Streptococci from Cattle.

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It was recorded by Minett, Stableforth, and Edwards (1929) that of the streptococcus strains isolated from 82 clinical cases of bovine mastitis 52 could be classed as hæmolytic and 30 as non-hæmolytic. The characters of the latter were somewhat variable, but the strains fell into two fairly distinct groups. The isolation of streptococci capable of fermenting mannite and inulin was also mentioned, but at that stage there was no satisfactory evidence that these were related to pathological change in the udder. The main object of the work recorded in this paper has been to define more clearly the cultural and biochemical characters of non-hæmolytic streptococci met with in udder milk, with special reference to those associated with mastitis. The serological characters of these and other mastitis strains will be set out in a later paper.

Isolation of Strains and Methods of Examination.

Most of the present series of 107 strains were isolated from milk samples of cows belonging to seven different herds. As milk samples from these cows were being examined at the Institute at regular intervals for another purpose, there was a good opportunity to study the relation between the variety of streptococcus present and the condition of the gland. Attention was not confined, however, to visibly altered milk because it was desired to obtain a comprehensive view of the non-hæmolytic streptococci which occur in the udder.

The technique of isolation was similar to that employed in all routine milk examination at this Institute, namely, the blood agar plate method. When the milk was normal in appearance 10 c.c. amounts were centrifuged and the deposits plated in serial dilutions in 5 per cent. ox blood agar. If the milk was visibly altered one or two drops of uncentrifuged material were used for plating. The character of the hæmolysis produced by deep colonies was noted after 24 and 48 hours at 37° C.

The strains described as non-hæmolytic in the present series include the gamma and alpha types of J. H. Brown (1919), and in many cases the deep colonies were surrounded by a green zone. It was realised, however, that the appearance of colonies in the original plates might not denote their hæmolytic character in the sense of

Brown, because for this plates containing not more than about 200 colonies should be examined. The main point to be noted is that no strain showed under a low magnification clear zones of hæmolysis of Brown's beta-hæmolytic type.

After 48 hours incubation in blood agar well isolated colonies were picked into 1 per cent. serum broth. After 18 hours in this medium the characters of the growth were noted and smears were stained by carbol thionin blue. In describing the morphological appearance both length of chain and size of coccus were taken into consideration. The serum broth culture was used for the following differential tests.

1.—Deep cultivation in *ox blood agar* plates.—The nature of hæmolysis produced, both in plates where colonies were numerous and where they were well isolated, was noted after 48 hours incubation at 37° C., and again after refrigeration for 48 hours.

2.—*Final Hydrogen-ion Concentration* in 1 per cent. glucose broth (Avery and Cullen, 1919).—Readings were made after five days at 37° C.

3.—*Hydrolysis of Sodium Hippurate.*— The glucose-peptone-pepsin medium of Ayers and Rupp (1922) was used in preference to their meat infusion medium. For the rapid precipitation of benzoate, 0·2 to 0·3 c.c. of 7 per cent. ferric chloride was found to be an optimal amount to add to 1 c.c. of five day culture. The tube in which the test is made should be shaken immediately the reagent is added.

4.—*Growth in Litmus Milk at 37° C.*—Tubes measuring 13 by 1·3 cm. were filled to a height of 6 cm. with whole milk suitably tinted with litmus. Observations as to acidification, clotting, reduction, and peptonisation were made daily for five days.

5.—*Growth in Litmus Milk at 10° C.*—During five days observation it was ensured that the temperature did not rise more than 1° or 2° C.

6.—*Reduction of Methylene Blue in Milk.*—The dye was added to whole milk to give a final concentration of 1 : 20,000, as this was found to be suitable for differentiation.

7.—*Reduction of Janus Green and Ammonium Molybdate.*—Tests were carried out as indicated by Ayers, Johnson, and Mudge (1924), except that 5 c.c. amounts of glucose broth were used for growing the culture. In both cases readings were made six and 18 hours after adding the reagents.

8.—*Fermentation Reactions.*—The basic medium was 1 per cent. peptone water, enriched with one-tenth of its volume of infusion broth and horse serum to a concentration of 1 per cent. The following test substances were added to give a concentration of 1 per cent.: lactose, saccharose, inulin, mannite, salicin, and raffinose. The final *p*H was noted after five days at 37° C.

9.—*Aesculin Fermentation.*—The medium of Harrison and Vanderleck (1909) was used, the results being taken after five days incubation. A blackening of the medium indicated splitting of æsculin.

10.—*Liquefaction of Gelatin.*—The sown medium was incubated at 37° C. for five days and then allowed to cool.

11.—*Resistance to Heat.*—Three drops of 18-hour broth culture were added to 5 c.c. glucose broth in 13 by 1·3 cm. tubes, and these were exposed for 30 minutes to temperature of 50° C. and 60° C. respectively. The tubes were then incubated at 37° C.

Characters of the Strains Examined.

By means of the above differential tests the strains could be arranged in four groups.

Group I.—Twenty-five strains. In blood agar plates showing after 48 hours incubation 1,000 or more colonies most of the colonies were surrounded by zones of partial hæmolysis. After refrigeration they were usually alpha-hæmolytic, a narrow ring of brownish corpuscles next to the colony being surrounded by a wider clear zone. In plates where the number of colonies was about 200 or less they were non hæmolytic or slightly green after incubation, and in this case there was no change on refrigeration. Growth in broth occurred as a granular or flocculent deposit with clear supernatant. On subcultivation the growth tended to become more diffuse. The chains were usually long and the individual cocci large and transversely elongated. Litmus milk at 37° C. was acidified and firmly coagulated within 48 hours with slight reduction at the bottom of the tube. At 10° C. there was no growth within five days. Methylene blue milk was not reduced, and tests with a few strains showed that the dye was bactericidal. The *p*H reached in glucose broth ranged from 4·4 to 4·7. Lactose, saccharose, and salicin were fermented, and hippurate was hydrolysed by every strain. There was no action on inulin, mannite, raffinose, or æsculin, and no reduction of janus green or ammonium molybdate. Gelatin was not liquefied. All strains resisted 50° C., but not 60° C.

Group II.—Twenty-two strains. After 48 hours incubation in blood agar colonies in congested plates were distinctly alpha-hæmolytic. The clear zone surrounding the fixed corpuscles was well marked and its width was increased by refrigeration. Isolated colonies after incubation were usually surrounded by a green zone, and refrigeration failed to produce any hæmolysis. Growth in broth appeared as a slight, finely powdered deposit with faintly turbid supernatant. The chains were short or of medium length and the cocci round and rather small. The changes in litmus milk at 37° C. were somewhat variable. About half the strains produced slight acidification without clotting, while the remainder caused clotting within 48 hours with reduction of the litmus in the lower two-thirds of the medium. On further incubation, the reduction disappeared until by the fifth day the clot was completely reddened. At 10° C. there was no growth. Methylene blue milk was reduced within 48 hours and the medium generally coagulated. The final *p*H in glucose broth varied between 5·0 and 5·3, and in no instance fell below 5·0. The only other substances fermented were lactose and saccharose. Hippurate was not hydrolysed by any of the strains, nor did they split æsculin or liquefy gelatin; janus green and ammonium molybdate were not reduced. All the strains resisted 50° C. but were destroyed at 60° C.

Group III.—Thirty-three strains. After 48 hours in blood agar the colonies were non-hæmolytic both in congested plates and in plates where they were well isolated. Broth cultures showed a slight

uniform turbidity after 18 hours incubation, growth being composed of diplococci and short chains of small round cocci. The action on litmus milk at 37° C. was somewhat similar to that of Group II strains, while at 10° C. the medium became slightly acid. Methylene blue milk was reduced and usually coagulated within 48 hours. In glucose broth the final *p*H ranged between 4·5 and 4·8. Hippurate was invariably hydrolysed and the following sugars were fermented : lactose, saccharose, inulin, mannite, and salicin. There was no action on raffinose or æsculin. Gelatin was not liquefied, and janus green and ammonium molybdate were not reduced. All the strains resisted 50° C. but not 60° C.

Group IV.—Twenty-seven strains. The characters of this group were rather more variable than those of the three preceding groups. After 48 hours deep colonies in blood agar plates, when present in large numbers, were alpha-hæmolytic, but well-isolated colonies were usually surrounded by a rather wide greenish zone. The naked-eye and microscopic appearances in serum broth were the most uniform characters observed. In this medium the supernatant liquid remained clear, and the deposit was either large and flocculent or consisted of coarse flakes not easily broken up by shaking. Microscopically the chains were extremely long and interwoven, and contained at least a hundred elongated and well-separated diplococci. At 37° C. litmus milk was clotted and partially reduced after two days incubation ; at 10° C. growth was indicated by slight reduction at the bottom of the tube. Methylene blue milk was reduced and coagulated. The final *p*H in glucose broth was sometimes as low as 4·5, at other times as high as 5·3. Lactose and saccharose were fermented by all the strains ; mannite, salicin, and æsculin by none. Inulin and raffinose were fermented by 17 strains and raffinose alone by two. There was no hydrolysis of hippurate or liquefaction of gelatin ; janus green and ammonium molybdate were not reduced. All strains survived 50° C., but not 60° C.

Distinguishing Characters of the Four Streptococcus Groups.

The characteristic properties of the streptococci are set out in Table I. From this it can be seen that there are a number of tests of value for differentiation.

With regard to blood agar the differences between the groups were not sufficiently striking to be of value. All would be classed as non-hæmolytic, but in crowded plates Group II showed more definite alpha-hæmolysis than the others. The growth in broth was, of course, chiefly dependent on the length of chain. This character, however, was not constant, but as a supporting observation it was useful, especially in the case of Group IV strains. Litmus milk at 37° C. was unsatisfactory for differentiation. Although acidification and firm coagulation were constant in Group I, individual strains of Groups II and III produced different results when tested on separate occasions.

TABLE I.

CHARACTERS OF NON-HAEMOLYTIC STREPTOCCUS STRAINS.

Group	No. of Strains	Growth in broth.	Morphology.	Litmus milk		Final pH in 1 per cent. glucose broth.	Fermentation of :—							Sod. hippurate.	Gelatin liquef.	Reduction of			Heat Resistance.		Mc-Conkey
				37°C.	10°C.		Lactose.	Saccharose.	Inulin.	Mannite.	Salicin.	Raffinose.	Aesculin.			Methblue.	Janus green.	Amm. molyb.	50°C.	60°C.	
I.	25	Gran. or floc. dep., clear super.	Cocci large flat., chains long.	AC	—	4·4–4·7	+	+	—	—	+	—	—	+	—	—	—	—	+	—	—
II.	22	Powdery dep., sl. turb.	Cocci small round chains, med. or short.	A, or AC. part. R.	—	5·0–5·3	+	+	—	—	—	—	—	—	—	+	—	—	+	—	—
III.	33	Sl. diffuse turb.	Cocci small round, chains short.	do.	Sl. A.	4·5–4·8	+	+	+	+	+	—	—	+	—	+	—	—	+	—	—
IV.	27	Abund. floc. dep., clear super.	Cocci elong., chains very long.	AC. part R	Sl. R.	4·5–5·3	+	+	+17 −10	—	—	+19 −8	—	—	—	+	—	—	+	—	—
Str. lactis	51	Diffuse turb.	Diplococci oval.	Comp RC., P+	R.	4·4–4·6	+	+	—	+	+	—	+	−28 −23	+4 −47	+	+	+23 −28	+	+	+
	6	Sl. dep. and turb.	Cocci oval; chains med.	Comp RC.	do.	do.	+	—	—	—	+	—	—	—	—	+	+ sl.	—	+	—	—
Calf fæces.	7	Diffuse turb.	Diplococci oval.	do.	do.	do.	+	+	—	+	+	—	+	+	—	+	—	—	+	+	+

A = acid, C = clot, R = reduction, P = peptonisation.

This irregularity was considered to be due to the use of varying amounts of seed material. In order to test this, falling amounts of broth culture of five strains belonging to each of the three groups were sown into litmus milk, and the medium observed during five days at 37° C.

It was found that with Group I strains acidification and firm coagulation with slight reduction occurred within five days with the smallest inoculum used (one drop culture, diluted 1 : 100). With Group II and III strains, large inocula, *e.g.*, four drops from a Pasteur pipette, produced coagulation in 24 hours followed by reduction, which extended two-thirds of the way up the column. Oxidation quickly advanced from the surface, so that by the fifth day only a small part of the clot remained decolourised. Smaller amounts of culture up to one drop caused acidification of the medium without coagulation.

It was clear from this experiment that the changes in litmus milk produced by Group II and III strains depended upon the amount of seed material. Coagulation would only occur if the medium had been seeded with a relatively large number of organisms, and in this case reduction followed. With smaller numbers there was acidification only.

Slight acidification of litmus milk at 10° C. was a suggestive test in the case of Group III strains. Failure to reduce methylene blue milk (1 : 20,000) was characteristic of Group I, this behaviour distinguishing them from the other groups. A combination of the three substances, salicin, inulin, and mannite, was useful for classification. The action of Group I on salicin, and of Group III on inulin and mannite, has been found constant even after several subcultures and storage in the cold. Inability to utilise these substances and low acid production in glucose broth were distinguishing characters of Group II. The reactions of Group IV were not uniform, but fermentation of inulin and raffinose by more than half the strains was important. The main features of the group, however, were naked-eye and microscopic appearances of the growth in broth.

Relationship of the Four Non-Hæmolytic Groups to Mastitis.

Table II shows the condition of the secretion at the time when streptococci were isolated.

TABLE II.

Relalationship of the Strains to Changes in the Milk.

Group.	*Total number of Strains.*	*Number of Strains isolated from*		
		Grossly altered Milk.	*Milk with abnormal dep. and reaction.*	*Normal Milk.*
I.	25	14	7	4
II.	22	19	2	1
III.	33	9	18	6
IV.	27	0	0	27

The fact that the milk is normal in appearance at a time when streptococci are present does not, of course, imply that these organisms are non-pathogenic.

The biochemical properties of Group I strains are similar to those of the well-recognised group of mastitis streptococci to which the name *S. agalactiæ* has been applied. Although it happened that in the present series 14 of the 25 strains in Group I were isolated from grossly altered secretion, the examination of a large number of milk samples at the Institute is in agreement with the general experience that mastitis caused by streptococci of this group is usually of a chronic catarrhal nature, visible alteration in the milk occurring at certain stages only of the lactation period, and particularly near its termination.

The nature of the disease caused by Group II streptococci has been discussed in a preceding article by Minett, Stableforth, and Edwards (1932). Nearly all Group II strains in the present series were isolated from cases of clinically acute mastitis that were followed by more or less permanent damage to the affected quarter. A number were from Herd A, described in the article mentioned. In a few cases the acute reaction was of sufficient intensity to overcome the infection, and the milk quickly returned to normal. In such instances streptococci could be isolated only when the secretion was altered in character. One strain was isolated in pure culture from an abscess beneath the skin of a quarter infected with a streptococcus of a similar type.

Most of the Group III streptococci were isolated from milk showing definite abnormality. In the case of 18 strains the only changes were increased deposit and alkalinity, but nine strains were cultivated from secretion which was grossly altered.

Group IV streptococci were not associated with any change in the milk.

Since streptococci with characters similar to Groups II and III have not been commonly described in the past it was essential to establish their relationship to mastitis. For this purpose a detailed examination was made in the first place of 14 milk samples from cases of mastitis in which these groups were present. These cases were selected because the first blood agar plates sown from the secretion showed 500 to 1,000 streptococcus colonies of the same uniform appearance. So far as could be judged by naked-eye examination, streptococci were the only organisms present. From the first plate of each sample six colonies were picked and identified. The result was that in seven of the cases all the colonies were verified by biochemical tests as belonging to Group II, while in the remaining seven cases every colony belonged to Group III.

Pathogenic Effects.

Tests have been made on mice, rabbits, and goats, and on lactating cows by intramammary injection.

Mice.—Nineteen strains of Group I and eleven of Group III, injected intraperitoneally in 0·5 c.c. amounts of 18-hour serum broth culture, caused practically no mortality in seven days. With nine Group II strains inoculation of a similar dose caused death in twelve out of 32 mice within the same period.

Rabbits.—Dr. Minett has examined the pathogenic properties by intravenous injection of Group I and Group II strains shortly after their isolation. Ten Group I strains were tested in amounts of 2·5 c.c. broth culture and six Group II strains with 1 and 2 c.c. amounts. The latter usually caused death within seven days, while the Group I strains failed to kill.

Goats.—Mr. Stableforth found with four Group II strains that living cultures, injected intravenously, caused joint lesions during the course of immunisation experiments.

Intramammary Injection in Cows.—The cows used in these experiments were in full milk and were proved by repeated cultural examination to be free of streptococcus infection. The quarter to be used, after being emptied at the evening milking, was injected by means of a teat syphon with 1 c.c. 24-hour serum broth culture, mixed with a few c.c. of milk which had just been withdrawn. The injected material was then generally massaged into the gland. Next morning only a small quantity of milk was removed, but after this milking was carried out in the usual way. Samples were examined daily, and on a number of occasions at least three colonies appearing in blood agar plates were verified. In the case of Cows 1 and 2, strains belonging to different groups were injected at the same time. In the first experiment *Str. lactis* was used as a control. The results were briefly as follows :—

Cow 1. Quarter LH. Group I.—The injection failed to cause clinical mastitis, and there was only a slightly increased alkalinity and deposit in the milk lasting for four days. After this the secretion returned to normal, but the streptococci persisted in the milk in numbers of 100 to 500 per c.c. for 96 days, when the cow was disposed of.

Quarter RF. Group II.—On the first day, the quarter was greatly enlarged, the swelling being hard and slightly painful. Five days later the quarter was normal in size though distinctly indurated. The milk was greatly reduced in quantity and contained clots. After 21 days the quarter was dry.

Quarter RH. Str. lactis.—No disturbance beyond a slight deposit in the milk for the first four days. The organism disappeared from the milk on the third day.

Cow 2. Quarter RH. Group III.—On the second day the quarter was slightly enlarged. The milk was not visibly altered, but laboratory examination showed increased alkalinity and deposit. The course of the disease was chronic, and the streptococci persisted in the quarter in numbers of about 500 per c.c. of milk during a period of 47 days observation. During this time the milk occasionally contained clots.

Quarter RF. Group IV.—There was a slight increase in the amount of deposit on the first day, and after this the organisms could not be recovered. The cow continued to milk normally in this quarter.

Cow 3.—Quarter LF. Group III.—Two days after injection, the quarter was greatly swollen, tense and painful, and the cow's temperature was 105·6° F. The swelling disappeared in four days, leaving the quarter slightly atrophied. At this time it was possible to obtain only a small quantity of thick yellowish secretion from the quarter and drying took place rapidly.

Quarter RF. Group I. (Injection made 38 days after the experiment on the LF quarter.)—On the first day there was moderate enlargement and slight pain in the quarter, and the milk was yellowish and clotted. After five days the quarter and milk appeared normal, and after ten days the milk yield was restored to normal. In this instance the streptococcus was excreted in the milk for eight days only.

Cow 4.—Quarter LF. Str. lactis.—No disturbance beyond slight increase in deposit for four days. The organism disappeared on the third day.

Quarter RF. Group II. (Injection made 16 days after the experiment on the LF quarter.) On the first day the quarter was greatly enlarged and slightly painful and there was extensive œdema under the abdomen. The cow's temperature rose to 105·8° F. The milk was grossly altered and only about four ounces could be obtained. Drying took place rapidly.

The preceding experiments show that of the two Group I strains one caused persistent infection of the gland. The Group II strains caused severe reactions followed by early drying of the quarter. One Group III strain gave rise to an acute mastitis followed by rapid drying, while the other produced a more chronic type of disease. The Group IV strain and two *Str. lactis* strains were non-pathogenic.

Unfortunately, for reasons of economy it was not possible to test the individual strains on separate cows, but the results give information as to the relative pathogenicity of the groups.

Relationship of the Non-Hæmolytic Groups to Certain Saprophytic Streptococci.

In view of the fact that non-hæmolytic streptococci of a saprophytic nature may occur in milk to which they may gain access from such sources as cows' skin or fæces, and in view of the possibility that some of these saprophytic forms might be confused with the present groups, it was desired to make a study of their inter-relationship.

Str. lactis.

There is now general agreement that the organism bearing this name and present in souring milk is a streptococcus composed of ovoid cocci in pairs and short chains. Orla-Jensen (1919) defined the characters of *Str. lactis* as a diplococcus which coagulated litmus milk within 24 hours at 30° C., grew at 10° C., and resisted a temperature of 60° C. Acid was produced from glucose, lactose, maltose, mannite and salicin but not from inulin or raffinose. Saccharose was not usually fermented.

Sherman and Albus (1918) pointed out the distinguishing characters of *Str. pyogenes* and *Str. lactis.* Typical lactic streptococci completely reduced litmus or methylene blue milk at 37° C. with subsequent coagulation. Growth also took place at 10° C.

Ayres, Johnson and Mudge (1924) distinguished *Str. lactis* from the streptococci commonly causing mastitis by the fact that the former reduced janus green and ammonium molybdate.

Klimmer and Haupt (1927) confirmed the distinctions already mentioned.

Avery (1929) found that the majority of non-hæmolytic mastitis streptococci failed to grow in milk containing methylene blue in a concentration of 1 : 5,000, whereas nearly all saprophytic strains from dairy products reduced the dye at this strength.

Str. acidominimus.

This name was applied by Ayres and Mudge (1922) to a streptococcus obtained from udder milk. The organism grew in broth in the form of short chains, failed to alter litmus milk at 37° C., and produced little acid from fermentable substances, but hydrolysed sodium hippurate. Belenky and Popowa (1930) cultivated this streptococcus from the skins of calves and from vaccine lymph. Among the characters described are failure to reduce janus green and ammonium molybdate.

Str. kefir.

Originally isolated from cheese, this streptococcus was found by Ayres, Johnson and Mudge (1924) to predominate in milk in the early stages of souring. In agreement with Evans (1918) they found its most characteristic feature to be the production of gas in fermentable media. Litmus milk at 37° C. was acidified, and there was no reduction of methylene blue milk or janus green. Glucose, lactose, saccharose, and raffinose were fermented, but there was no action on salicin, mannite, inulin, or sodium hippurate.

Str. bovis.

First described by Orla-Jensen (1919) as the common streptococcus of cow fæces. It grows in broth as short chains, acidifies litmus milk, and ferments raffinose and inulin. It differs from *Str. kefir* in failing to produce gas from glucose. Ayers and Mudge (1923) found that 75 out of 78 streptococcus strains from the fæces of cows agreed with this type. Their strains fermented glucose, lactose, saccharose, and raffinose, and in addition 21 strains fermented inulin.

Str. viridans bovis.

Belenky (1930) cultivated this organism from vaccine lymph as well as from the skin, saliva, and nasal discharge of cattle. It grows in broth as a diplococcus, and acidifies litmus milk without coagulation. Mannite, raffinose and æsculin are fermented, and hippurate is generally hydrolysed. Some strains belonging to this species resist 60° C.

Comparing the characters of the present groups with the saprophytic streptococci named above, it is evident that the fermentation of raffinose and inulin by most of the Group IV strains brings them into line with *Str. bovis*, though growth in broth in the form of very long chains has not been mentioned in the case of the latter group. Again, it may be seen that in certain respects, such as short chain formation in broth and reduction of litmus and methylene blue milks, the mastitis Groups II and III are similar to *Str. lactis*.

Str. lactis is a common saprophyte in milk, and since some of its characters resemble those of the mastitis streptococci an examination has been made for comparison of 57 strains isolated from samples of udder milk which had been allowed to become sour at room temperature.

Str. Lactis Strains Examined.—The characters of 51 of these strains were in agreement with those of the well-recognised type

species. (See Table I). They produced colonies of the gamma type in ox blood agar and appeared as oval diplococci in broth. Litmus and methylene blue milks were completely reduced within 18 hours, followed by coagulation. Four strains peptonised the clot and the same strains liquefied gelatin. All the strains reduced litmus milk at 10° C., and resisted heat at 60° C. for 30 minutes. Lactose, saccharose, mannite, and salicin were fermented, but not inulin. Eight strains produced slight acid from raffinose and 28 hydrolysed sodium hippurate. Ammonium molybdate was reduced by 23 strain and janus green by all. Æsculin was split by the 51 strains.

Since Harrison and Vanderleck's æsculin medium contains sodium taurocholate, it seemed probable that a positive result with the æsculin test depended firstly upon the ability of lactis strains to grow in the presence of bile salt, and secondly on the fermentation of æsculin.

An experiment on this point was carried out by sowing ten strains belonging to each of the mastitis Groups I, II, and III and ten *Str. lactis* strains into (*a*) æsculin medium without bile salt, (*b*) æsculin medium containing 0·25 per cent. sodium taurocholate. The results showed that in medium (*a*) only Group III of the mastitis strains fermented æsculin, whereas in medium (*b*) there was no action by these strains. *Str. lactis* fermented æsculin in both media, this being obviously due primarily to the ability to grow in the presence of bile salt.

The remaining six strains from sour milk differed in the following respects. Growth in broth was slow, and smears showed chains of medium length composed of oval diplococci. Saccharose, mannite, and æsculin were not fermented, and janus green was only slightly reduced after 18 hours. They were destroyed by heat at 60° C. These six strains are somewhat similar to certain streptococci which may be found in milk " starters," *viz.*, *Str. cremoris*. The differentiation between *Str. cremoris* and *Str. lactis* is not clear, but it is generally held that slow growth in broth with chains of medium length and an inability to utilise saccharose or carbohydrates other than the monosaccharides are characteristic of *Str. cremoris*.

The main characters which serve to differentiate the non-hæmolytic mastitis Groups I, II and III from those of *Str. lactis* may be seen in Table I. Morphologically, the majority of the *Str. lactis* strains grew as oval diplococci, contrasting with the mastitis strains in which individual cocci were round and chain formation was present. In litmus milk at 37° C., the lactis strains, even with small seedings, showed their characteristic changes, *viz.*, rapid, complete reduction followed by coagulation with slow oxidation. This served to distinguish them from Groups II and III, where clotting followed by partial reduction was produced by large seedings only. The growth of *Str. lactis* at 10° C. with complete reduction was in contrast with the slight acid production by Group III at this temperature. The uniform failure of *Str. lactis* to ferment inulin was also a useful means of distinction from Group III. The ability of lactis strains to resist heat at 60° C., to reduce janus green, and to ferment æsculin were characters of value. Since the value of the æsculin test rested upon

the ability of *Str. lactis* to grow in the presence of 0·25 per cent. sodium taurocholate, the æsculin medium might be replaced with advantage by a more simple medium such as that of MacConkey.

Streptococci from Fæces.—In order to compare the characters of fæcal types with the mastitis strains, seven strains of streptococci from fæces of young calves were examined. From Table I it will be seen that their characters were generally similar to those of the main group of *Str. lactis*. All the strains hydrolysed sodium hippurate, but none reduced janus green or ammonium molybdate. It is now generally agreed that the characters of *Str. lactis* are similar to those of " enterococci " from human fæces, hence it would appear that the streptococci from calf fæces are similar to those occurring in human fæces. Orcutt (1926), Belenky and Popowa (1930) also cultivated from calf fæces strains related to human enterococci.

Discussion.

Of the non-hæmolytic streptococci described, those falling into Group I may be taken as representative of the group of mastitis streptococci to which the name *Str. agalactiæ* has been given in the past. Actually, the majority of the strains examined were from cases of chronic catarrhal mastitis, the form of the disease with which *Str. agalactiæ* is now well recognised to be associated.

It is apparent from a survey of the more recent literature that non-hæmolytic streptococci with characters other than those of *Str. agalactiæ* have been occasionally isolated from cases of mastitis.

Jones (1918) examined 39 strains of non-hæmolytic mastitis streptococci. Thirty-four fell into a single group with characters similar to those of Group I above, and five strains were different in giving slight turbidity and short chains in broth, partial clot in litmus milk and low acid production from sugars.

Ayers and Mudge (1922) investigated 20 strains of non-hæmolytic udder streptococci. Of these, 15 were similar to Group I, while five differed in reducing methylene blue milk, producing acidification only in litmus milk, and fermenting mannite.

Rudolf (1926), who used litmus milk for differentiation, cultivated from mastitis secretion three strains of streptococci which reduced the medium and grew as short chains. These organisms were looked upon as the cause of the mastitis, and were considered to be *Str. lactis* strains which had increased in virulence.

v. Sande (1927) also concluded that in certain circumstances *Str. lactis* could become pathogenic, because 15 out of 29 streptococcus strains isolated from cows with mastitis were thought to behave like *Str. lactis* in litmus milk.

Klimmer, Haupt and Roots (1928) reported on the examination of 201 streptococcus strains isolated from normal and from altered milks. Of these, 180 corresponded to *Str. agalactiæ* and the remaining 21, which were isolated from a single cow affected with mastitis, fell into a second group possessing the following characters : slow acidification of litmus milk at 37° C. without coagulation, reduction of methylene blue milk, final *p*H of 5·0 to 5·3 in lactose broth, and slight growth at 10° C.

Diernhofer (1930) cultivated twelve strains (Group III C) from one quarter of a cow with mastitis. They were distinguished from *S. agalactiæ* by the following characters: growth in broth as short diplococcal chains, coagulation and reduction of litmus milk, reduction of methylene blue milk, final *p*H in lactose broth of 4·8 to 5·1, and failure to hydrolyse sodium hippurate. The injection of one strain into the quarter of a healthy cow caused an acute mastitis with atrophy of the quarter.

Seelemann (1932) reports that on a number of occasions since 1930 he has cultivated, from the secretion of isolated cases of chronic mastitis, streptococci which he classifies as "atypical." It appears to be extremely doubtful, however, whether these should be excluded from the *S. agalactiæ* group. On various occasions Seelemann has grown from the altered milk in mastitis two other groups which were characterised by short chains in broth; one of the groups produced slight acidification only in litmus milk while the other behaved like *Str. lactis* in this medium at 37° C. and at 10° C.

The characters of certain of the less common streptococci mentioned in the above survey are suggestive of those of Groups II and III of the present series. Rudolf and v. Sande, from their results with litmus milk, concluded that they were dealing with *Str. lactis*, but the evidence is insufficient to justify this view. It has been shown that in this medium, under certain conditions, streptococci of Groups II and III can produce changes simulating those of *Str. lactis*, but other tests show this group to be quite distinct. Among the large numbers of samples of udder milk which have been examined at this Institute during the last three or four years, *Str. lactis* has been found on rare occasions only, indicating that it is not a normal inhabitant of the udder. The saprophytic nature of *Str. lactis* is also indicated by its rapid disappearance from the udder after small amounts of culture have been injected.

The nature of the disease caused by Group II streptococci has been discussed in a preceding paper (Minett, Stableforth and Edwards, 1932). Most of the strains in the present series were isolated from cases of acute mastitis, in which as a rule there was some permanent damage to the affected quarter. In many of these cases the streptococci were present in large and predominating numbers in the altered secretion. The streptococci of this group were found to be relatively pathogenic for mice, rabbits, and goats, whereas Group I streptococci were of low virulence. It appears therefore that on account of this low virulence Group I streptococci provoke only a slight response in the udder tissue and are, therefore, well adapted for causing chronic mastitis. When for some reason these streptococci suddenly increase in number the reaction may become severe. On the other hand, the more virulent Group II streptococci generally give rise to a severe reaction evidenced by an acute mastitis, which, according to the degree of infection either leads to rapid disappearance of infection and repair of the tissues or to complete destruction of the gland. These inferences are supported by the results of experimentally inoculating two strains of each group into the healthy udder.

Most of the Group III streptococci were isolated from slightly altered milk, and a few from secretion which was grossly altered. It is probable that strains of this group are, as a rule, of low pathogenicity, although one strain produced a severe mastitis on intramammary injection.

Streptococci belonging to Group IV were not found to be associated with disease and one strain failed to cause disturbance when injected into the udder of a healthy cow. Although the biochemical characters of Group IV were not quite uniform, there is a resemblance in some respects to the fæcal group described as *Str. bovis*.

Summary.

One hundred and seven strains of non-hæmolytic streptococci were isolated from the same number of milk samples. By cultural and biochemical examination these strains fell into four groups of which the main characters of differential value were the following :—

Group I.—Twenty-five strains. Long chains of transversely elongated cocci in broth ; acid and firm clot in litmus milk ; no reduction of methylene blue milk (1 : 20,000) ; final *p*H of 4·4 to 4·7 in glucose broth ; fermentation of salicin and not of mannite, inulin, or raffinose ; hydrolysis of sodium hippurate. These are the characters of the group referred to as *Str. agalactiæ*.

Group II.—Twenty-two strains. Short to medium chains of small rounded cocci in broth ; acid, or acid with clot, and partial reduction in litmus milk ; reduction of methylene blue milk ; final *p*H of 5·0 to 5·3 in glucose broth ; no fermentation of salicin, mannite, inulin, or raffinose ; no hydrolysis of hippurate.

Group III.—Thirty-three strains. Short chains of small round cocci in broth ; acid, or acid with clot, and partial reduction in litmus milk at 37° C. ; slight growth in milk at 10° C. ; final *p*H of 4·5 to 4·8 in glucose broth ; fermentation of salicin, mannite, and inulin but not raffinose ; hydrolysis of hippurate.

Group IV.—Twenty-seven strains. Abundant flocculent deposit in broth, composed of extremely long chains of elongated cocci ; fermentation of inulin and raffinose by more than half the strains. In their fermentation characters these strains fall into the group of bovine fæcal streptococci, to which the name *Str. bovis* has been assigned.

The changes produced in litmus milk with Groups II and III streptococci varied with the amount of seed material. Small inocula caused acidification without coagulation.

The relationship of these four groups to bovine mastitis has been discussed. Most of the Group I strains were isolated from the milk samples of cows with chronic catarrhal mastitis, and one of these strains set up similar changes when injected into the udder of a lactating cow. Group II strains were isolated from more acute cases of mastitis. A number of these strains were found to be pathogenic for mice, rabbits, and goats, and two strains on intramammary injection in cows led to acute changes with permanent damage and followed by early drying. Group III strains were cultivated from milk showing definite abnormality, though in the majority of cases

the changes were slight. Cultures of two strains, injected into normal udders of cows, caused mastitis, which in one case was acute and in the other chronic. Group IV strains were non-pathogenic and there is no evidence of their association with mastitis.

The mastitis Groups I, II and III could be distinguished by certain properties from *Str. lactis* and other saprophytic streptococci which may be met with in milk. Among the latter, reference has been made to groups to which the following names have been applied : *Str. acidominimus*, *Str. kefir*, *Str. bovis*, and *Str. viridans bovis*. Among the differential points of value in the case of *Str. lactis* are its morphology, growth in litmus milk at 10° C., resistance to heat at 60° C., reduction of janus green, and fermentation of æsculin. Positive results with the æsculin test are primarily due to organisms tolerating bile salt.

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ORGANISMS OF THE B. INFLUENZÆ GROUP IN FOWLS.

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Although bacteria of the influenza group have been found in some animals, comparatively little is known of their distribution, growth requirements, cultural characters, and pathogenicity. They are of interest not only as possible causes of disease in animals but also when compared with strains isolated from human sources. The whole influenza group of bacteria is somewhat diverse in morphology, pathogenicity, and antigenic structure (Scott, 1930), but on the basis of dependence for growth upon the X and V factors three divisions are recognised : I. strains requiring both X and V factors (*B. influenzæ*, *B. Koch-Weeks*), II. strains requiring the X factor only (*B. hæmoglobinophilus canis*), III. strains requiring the V factor only. This classification is probably the most satisfactory at present available, as other criteria are variable, *e.g.*, strains of *B. influenzæ* vary as regards hæmolysis, indole production, and pathogenicity. Rosher (1931), however, has noted that most indole-producing strains of *B. influenzæ* were pathogenic for mice, whereas most of those that did not form indole were non-pathogenic.

The term *B. parainfluenzæ* has been applied to bacteria of division III. that were non-hæmolytic, but hæmolytic strains dependent upon the V factor only have been described.

Little importance attaches to differences in morphology. *B. influenzæ* in exudates is characteristically a small cocco-bacillus. In culture it may retain this appearance, may form slender rods and long filaments, or may become markedly pleomorphic. The morphology of any one strain is, however, usually fairly constant. The antigenic structure of this group is markedly diverse. The majority of strains do not cross-agglutinate and identification or classification has not so far been possible by serological tests.

The distribution of these organisms in animals has not been investigated on a large scale and consequently little information is available. Oslen (1911) recovered from the pneumonic lungs of dogs suffering from distemper bacteria resembling *B. influenzæ*, but as their growth requirements were not determined it is now not possible to be certain of their exact nature. Kristensen (1922), from the mouth of five guinea-pigs of 60 examined, isolated organisms belonging to the influenza group. They were said to be morphologically atypical but there are no further details which would relate them to other strains. He failed to find similar organisms in 43 mice and 33 horses. Rivers and Bayne-Jones (1923) described seven strains from the throat of cats. Six of these corresponded to *B. parainfluenzæ*, being non-hæmolytic and requiring the V factor only. One strain resembled *B. influenzæ* in requiring both factors. De Blieck (1931) isolated from the respiratory passages

of fowls affected with coryza an organism resembling *B. influenzæ* but did not determine its growth requirements. Lewis and Shope (1931) isolated a hæmophilic organism, *H. influenzæ suis*, from the respiratory tract, heart blood, and spleen of swine spontaneously and experimentally infected with swine influenza. They state that the organism required both X and V factors and was indistinguishable morphologically and culturally from the non-indole producing strains of *B. influenzæ*. They failed to isolate similar organisms from swine free from influenza. Attempts to reproduce the disease by inoculation of cultures failed. In further experiments Shope (1931) showed that inoculation of bacteria-free filtrates of infective material into swine caused only a mild afebrile disease but that inoculations of cultures along with filtrates reproduced a disease identical clinically and pathologically with swine influenza.

The present communication deals with observations made on eleven strains belonging to the influenza group which were isolated from the respiratory passages of nine fowls. Seven strains were from six birds out of a flock in which there was an outbreak of coryza ; the remaining four strains were obtained from three birds from other flocks. The following table indicates the origin of the strains.

Table Showing the Source of the Strains.

Strain.	*Source.*	*Clinical Condition.*	*No. of Fowl.*
L1	Trachea	Coryza	1
L2	Larynx	Coryza	2
L3	Larynx	Coryza	3
L4	Larynx	Coryza	4
H5	Larynx	Coryza	5
H6	Larynx	Coryza	6
H7	Nasal Discharge	Coryza	4
L8	Larynx	Leukæmia	7
H9	Larynx	Healthy	8
L10	Larynx	Fowl Paralysis	9
H11	Larynx	Fowl Paralysis	9

L= light growth. H=heavy growth.
L4 and H7 were isolated from one bird, No. 4, and L10 and H11 from Fowl 9.

History of the Outbreak.

The condition was observed in a flock of 200 pullets about six months old bought from a dealer in October, 1930. A few days after arrival at the farm several birds showed signs of acute coryza, *viz.*, mucoid discharges from the eyes and nostrils and respiratory distress. Within a week about half of the flock was affected but not more than 20 birds died. The survivors remained in poor condition for some months and the egg yield was extremely small, about three eggs a day from 180 pullets. Eight birds were killed and examined at intervals during the next two months.

Clinical Signs.

All the birds were emaciated and the combs and wattles were pale and shrunken ; in two there were mucoid discharges from the

nostrils and in one also a mucoid discharge from one eye. The first two birds received showed considerable respiratory distress but this sign was not so prominent in those received later. The temperature of all birds was normal.

Post-mortem Lesions.

In two birds there were large caseous masses in the nasal chambers, caseous nodules adhering to the mucous membrane of the mouth and pharynx, and caseous casts filling the bronchioles of the anterior portion of the lungs.

In another bird the mucous membrane of the trachea was inflamed and covered with a blood-tinged mucoid exudate.

In four birds there were flakes of sticky yellow purulent material adhering to the membranes of the thoracic and abdominal air-sacs. The liver, spleen, kidneys, and other organs were normal in all eight birds.

Bacteriological Examination.

Films of the caseous purulent material in the thoracic and abdominal air sacs were stained by Gram's method and the Ziehl-Neelsen method but no bacteria were seen. Cultures made on blood-agar plates of this material from four birds remained sterile. Cultures of the caseous matter in the nasal chambers yielded a growth of a coliform bacillus. Blood cultures made from two live birds (5 c.c. of blood in 50 c.c. of broth) remained sterile. Cultures were made on blood agar from the blood-stained exudate in the trachea of one bird and from these plates strain one was isolated. Five more birds from the affected flock and four birds from other flocks were examined in the manner described below. Two of the birds affected with coryza were examined more fully ; cultures made from the lower portion of the trachea, lungs, liver, spleen, ovary, bone-marrow, and blood were negative. No bacteria resembling *B. influenzæ* were recovered from one of the two healthy birds examined.

Isolation of Strains.

A swab that had been passed into the larynx was used to inoculate a plate of blood agar and one of Fildes' agar. Any small colonies resembling those of *B. influenzæ* which appeared after 24 or 48 hours incubation at 37° C. were subcultured to plates of Fildes' agar and to plates of blood agar that had been inoculated at two or three points with an air-coccus. After 24 hours incubation at 37° C. the cultures were filmed and stained by Gram's method. Those which were Gram-negative bacilli were subcultured into Hartley's broth, serum agar, and fresh blood agar plates inoculated at several points with an air-coccus. The cultures which failed to grow in Hartley's broth and serum agar but which showed satellitism on the blood agar plates were then tested for growth requirements.

Morphology.

All strains were Gram-negative, non-motile, and not capsulated. Some strains were composed entirely of cocco-bacilli, others of short rods with occasional filaments and long twisted forms. The cocco-bacilli often stained poorly.

Cultural Characters.

No growth was obtained on hormone agar or serum agar; on blood agar it was scanty and the colonies were very small. The most luxuriant cultures were obtained on hormone agar or Fildes' agar plus 20 per cent. of yeast extract prepared according to the method of ThjØtta and Avery (1921). All the strains showed the phenomenon of satellitism with an air-coccus.

The eleven strains could be divided into two groups, H and L (*see* table), differing in the size of colonies and in the amount of growth in fluid media. The H strains (5, 6, 7, 9, 11) grew profusely on hormone agar or Fildes' agar with yeast extract and about colonies of an air-coccus on blood agar. The first two of these media furnished the most copious growth. On them, after incubation for 24 hours, the colonies were about 1 mm. in diameter, opaque, creamy in colour, raised with entire edges and smooth surfaces, moist and glistening, and butyrous in consistency. With one exception, *viz.*, strain H11, they were easy to emulsify. Colonies of strain H11 mixed with water or saline formed clumps which were extremely difficult to spread on a glass slide. After 48 hours the colonies were about 2 mm. in diameter, and on moist or heavily inoculated yeast agar plates heavy confluent yellowish growths with more opaque patches here and there were obtained. The addition of yeast extract to the Fildes' agar seemed to improve the growth and a similar observation was made with heated blood agar.

The six L strains (1, 2, 3, 4, 8, 10) grew very poorly on all media that were tested. Yeast agar, hormone agar inoculated with a coccus, boiled blood agar, and even Fildes' agar with yeast extract, have all proved unreliable and at best yielded very scanty growth. The most satisfactory medium for maintaining the cultures was found to be fresh blood agar inoculated at two or three points with an air-coccus. On yeast agar and on Fildes' agar with yeast extract after 24 hours incubation the colonies were very small, less than 0·5 mm. in diameter, transparent, with entire edges, smooth, moist and glistening. After 48 hours the colonies had increased slightly in size. In the case of strain L4 they became white and more opaque. If the plate was moist when inoculated a faint transparent confluent growth was sometimes obtained, but usually the colonies remained discrete. On Fildes' agar which was quite satisfactory for *B. influenzæ* growth often failed.

In peptone water, hormone broth, or Hartley's broth to which 20 per cent. yeast extract had been added, the H strains grew more profusely. After five to six hours there was a marked turbidity

which continued to increase, and to form a deposit after 24 hours. One strain H11 differed in forming from the beginning of the culture coarse granules which sank, the cultures consisting of a granular deposit in a clear fluid.

All the L strains except L3 formed after 24 to 48 hours a faint even turbidity which settled after two days to form a faint granular deposit. Strain L3 formed little or no turbidity but instead a faint fluffy deposit which broke up on shaking.

All strains were non-hæmolytic on blood agar.

Indole was not formed in peptone water cultures with yeast extract or Fildes' digest added. The methods of testing for indole were :—

(1) Holman and Gonzales' (1923) method, *i.e.*, suspending above the cultures strips of filter paper which have been soaked in saturated watery solution of oxalic acid and dried. In this test the formation of indole is detected by the appearance of a pink coloration of the paper.

(2) By the use of Böhme's reagent, *i.e.*, paradimethylaminobenzaldehyde on cultures 24 hours, 48 hours, and five days old.

Fermentation of Carbohydrates.

Tests for the fermentation of carbohydrates were made by inoculating several drops of a young yeast broth culture into tubes of 1 per cent. solutions of various carbohydrates in 1 per cent. peptone water with yeast extract added. The *p*H of the solutions was 7·5 and Andrade's indicator (rosanilin hydrochloride) was used. Tubes of 1 per cent. peptone solution with yeast extract and indicator were inoculated at the same time, as controls.

Fermentation reactions for this group have been described by various authors, but as Kristensen (1922) has noted that *B. influenzæ* has the property of forming acid in solutions containing protein and that the acidity may be as great as in similar solutions containing carbohydrates, the fermentation reactions that have been recorded require careful scrutiny in the light of this fact. As H strains produced acid in the control tubes we do not feel warranted in making any conclusions on these fermentation tests. The L strains grew poorly and produced no change in reaction either in the control tubes or in those containing carbohydrates after incubation for seven days. Glucose, maltose, mannite, lactose, sucrose, and salicin were tested.

In *Litmus Milk* with yeast extract the H strains produced slight acid but caused no coagulation. With L strains no change was observed.

Reduction in Nitrates.

All the strains except L10 and H11 reduced nitrates to nitrites. No stress is laid on these exceptions. The result may have been due to scanty growth.

Bile-Solubility.

Sellards and Sturm (1919), Neufeld and Etinger-Tulczynska (1930), and Pittman (1931) have shown that *B. influenzæ* is bile-soluble. Bile-solubility tests of the strains of Group I were made by adding a few drops of acetone-choleic acid (Downie, Stent and White, 1931) to about 1 c.c. of a 16 hours yeast broth culture, the reaction of the culture having been adjusted to *p*H 7·5. All the II strains were found to be bile-soluble.

Growth Requirements.

In their dependence upon accessory substance for growth all strains were alike ; all required the V factor only and grew readily in the absence of the X factor.

Tests were made according to the method of Fildes (1923) by inoculating the four following media with a loopful of a bacterial suspension made by emulsifying the amount of growth from a solid medium that would adhere to the point of a platinum needle in 8 to 10 c.c. of saline, which avoided carrying over any accessory substances in the inoculum : (*a*) 1 per cent. peptone solution ; (*b*) peptone solution with 0·1 c.c. of 1 per cent. solution of hæmatin (British Drug Houses) in 4 c.c. of medium ; (*c*) peptone solution containing 20 per cent. of yeast extract ; (*d*) peptone solution with hæmatin and yeast extract. After incubation for 24 hours (and 48 hours when necessary) subcultures were made from each tube to a suitable solid medium. Growth in subculture provided evidence of multiplication in the test media. All strains grew in the media (*c*) and (*d*) above but not in (*a*) or (*b*), indicating that they required the V factor only. These tests were made on three occasions over a period of six months and have not varied.

Pathogenicity.

There is no question that *B. influenzæ* is pathogenic for man and that some strains are pathogenic for mice, but the pathogenicity of organisms of this group for domestic animals and fowls is still unsettled. In man they are very frequently found in the mouths of healthy persons, Fleming and McLean (1930) having demonstrated them in all of 30 adults examined. Their prevalence in the naso-pharyngeal flora of man is, however, subject to wide variation ; they are sometimes easy to demonstrate and at other times can only be isolated by special selective methods (Fleming and McLean, 1930).

De Blieck (1931), as already mentioned, is the only author who has recorded the presence of organisms of this group in fowls, and he has suggested that they were the cause of coryza in the birds he examined because of their presence in the exudate and their pathogenicity for fowls after passage through a series of fowls.

The pathogenicity of six strains for mice and fowls was tested, as follows :—

(*a*) Strains L1, H5, H6, H7 and H11 were grown on Fildes' agar for 24 hours. The growth from three slopes was suspended in 5 c.c.

of saline and 0·5 c.c. inoculated intraperitoneally into each of two mice. The mice inoculated with strains H5 and H7 died within 24 hours, the others remained well.

(*b*) At a later date strains H5, H6, H7 and L4 were tested. In this experiment the suspensions were standardised, and, as Rosher (1931) has shown that the inclusion of a small quantity of X and V growth factors in the inoculum definitely increases the virulence of influenza bacilli for mice, a small quantity of Fildes' digest was added to the suspensions. Cultures grown for 24 hours on yeast agar plates were washed off with broth and standardised to about 7,000 million organisms per c.c. To 2·5 c.c. of each suspension 0·25 c.c. of Fildes' digest was added, and 0·5 c.c. of this mixture was inoculated intraperitoneally, four mice being used for each suspension. The suspension of H7 without the addition of Fildes' digest was similarly inoculated into four mice.

Strains H6 and L4 were not pathogenic. Strain H7 (with the addition of digest) killed three mice within 72 hours, this result confirming the pathogenicity of the strain as shown in the first experiment. The suspension without digest killed only one mouse, suggesting a confirmation of Rosher's observations. Strain H5 killed one mouse ; it was also pathogenic in the first experiment.

On post-mortem examination there was excess of peritoneal fluid, enlargement and congestion of the lymph glands, and in some cases enlargement and congestion of the spleen. The organism was recovered from the blood and spleen in all cases but not from the lymph glands.

(*c*) Three chicks about ten days old were inoculated subcutaneously with 0·5 c.c., 0·25 c.c., and 0·1 c.c. respectively of a 24 hours culture in Fildes' broth of strain H5. All remained well. One adult fowl inoculated intravenously with 0·5 c.c. of the same culture also remained well.

One adult fowl was inoculated intravenously with 0·5 c.c. of the suspension of strain H7 which was used for experiment (*b*). It remained well.

The conclusion may be made that strains H5 and H7 were pathogenic for mice. Both strains belonged to the group that grew profusely. It is of interest that strain L4 isolated from the same bird as H7 was not pathogenic for mice and differed from it also in growing poorly in media.

The reason for the lack of pathogenicity of these two strains for fowls is not evident. Not many tests were made, and these only after the strains had been isolated for at least three months. The tests would have been of more value if they had been made with freshly isolated cultures.

That strains H5 and H7 possessed invasive properties was indicated by recovering them from the blood and spleen. Also a filtrate of an 18 hours yeast broth culture of strain H7 did not kill mice when 1·0 c.c. was inoculated intraperitoneally.

Discussion.

Relation of the Bacteria Isolated to B. Influenzæ.

In growth requirements (*i.e.*, dependence upon the V factor only), non-production of hæmolysin, and non-production of indole, the bacteria described correspond to the organisms isolated from man by Rivers (1922) and from cats by Rivers and Bayne-Jones (1923), and termed by them *B. parainfluenzæ*. One of their strains required the incorporation of unaltered protein in the medium and in this respect the L strains would appear to resemble it, their scanty growth on Fildes' agar and chocolate agar and the improvement resulting from the addition of yeast extract to these media indicating marked dependence on the V factor. The moderate growth of H strains on blood agar, as compared with hormone agar, in symbiosis with air cocci may be explained by the observations of Davis (1921) and Knorr (1924), who noted that fresh serum had an inhibitory effect on the V factor.

Relation of the Bacteria Isolated to Coryza in Fowls.

Without further experimental work no definite opinion can be formed of the significance of these organisms in the outbreak of coryza described. The points in favour of them being concerned in the disease are that they were present in the respiratory passages of the affected fowls in much greater number than in the healthy bird and other birds examined. There is, however, some indication that they are not infrequent in the normal upper respiratory tract of fowls. Only four such fowls were examined, but organisms were isolated from three, and from each of two birds two distinct strains were obtained.

Summary.

1.—The isolation of an organism of the influenza group from the upper respiratory tract of fowls is recorded. In its dependence for growth on the V factor only, absence of hæmolysin, and failure to produce indole, it corresponds to the *B. parainfluenzæ* (so-called) isolated from man by Rivers and from the throat of cats by Rivers and Bayne-Jones.

2.—Seven strains were isolated from six birds suffering from inflammation of the respiratory tract which had occurred as an outbreak in a flock. Four strains were obtained from the larynx of three birds with no abnormality of the upper respiratory tract. One normal fowl did not yield a culture.

3.—All the strains tested were soluble in acetone-choleic acid and produced acid from peptone.

4.—Minor differences in cultural characters and in morphology are discussed.

5.—Some of the strains isolated were pathogenic for mice and possessed invasive properties.

6.—The significance of these organisms as the cause of the outbreak is considered to be uncertain.

7.—It is probable that bacteria of the influenza group are not infrequently present in the upper respiratory tract of normal fowls.

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STUDIES IN THE HISTOPATHOLOGY OF LOUPING-ILL.

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Introduction.

During the years 1929-31 it has been shown that a filterable virus present in the central nervous system is the etiological factor of louping-ill and that this virus is capable of being transmitted in series in sheep by intracerebral inoculation of preparations of brain and cord obtained from cases of the disease. It was further found that the pig and the mouse were susceptible to experimental inoculation. The results of these investigations have previously been published (1, 2, and 3).

In our field investigation a number of cases were met with showing symptoms which were at that time recognised to be those of louping-ill, but post-mortem examination showed that such cases could represent different pathological entities and we were able to make a classification similar to that of M'Fadyean (4). This author concluded that the principal morbid conditions grouped under the name of louping-ill were :—

(I) Pyæmic spinal meningitis caused by pyogenic bacteria.
(II) Gastritis and enteritis from indigestible substances (wool, sand, dried grass) in the stomach or intestines.
(III) Disorders of brain functions, paralysis and general weakness, with, in some cases, excess of cerebro-spinal fluid in the cranial cavity, but without gross lesions in any of the organs of the body.

In our work material was taken for transmission experiments and for histological examination from cases belonging to Group III.

Review of the Literature.

Subsequent to the publication of M'Fadyean's work, M'Gowan and Rettie (5) and Stockman (6) gave accounts of the histopathology of the condition without having been able definitely to associate the lesions which they found with the action of any specific pathogenic agent. M'Gowan and Rettie say :—

" In the acute stage of the disease we have found the pia mater and the grey and white matter of the brain infiltrated with small round cells. The vessels of the brain and cord are engorged with blood and their perivascular sheaths are infiltrated with small round cells, cells with a clearer round nucleus and a larger amount of protoplasm, and cells with deeply indented nuclei which in some cases almost resemble polymorphonuclear nuclei.

In the chronic stage, the pia mater, nerve roots, the grey and white matter' and the perivascular sheaths of the vessels are infiltrated with the small round cell and with cells probably derived from proliferation of connective-tissue cells. The nerve cells, especially of the anterior horns of the spinal cord, are in all stages of disintegration, from conditions of chromatolysis, pyknosis, excentricity of nuclei, to entire disappearance and replacement by neuronophages."

Stockman found distinct microscopic lesions in the brain and spinal cord of cases which he regarded as typical of louping-ill :—

" Generally speaking, it may be said that the lesions vary from congestion of the small vessels, with or without small hæmorrhages into the surrounding tissues, to more or less intense inflammation marked by passing of the white cells of the blood through the vessel walls in to the sheaths and nerve tissues. When this cell infiltration of the tissue occurs, the inflammatory cells show up in stained preparations as dense groups of nuclei, or they may be diffused in much smaller numbers through the tissues. These inflammatory phenomena are not by any means confined to the grey matter, or to any part of it, but as a rule, they are more prominent in the grey matter, possibly because the tissue is highly vascularised with small vessels. The central canal of the cord in the grey matter may contain a coagulum with or without inflammatory cells, usually without. The epithelial cells lining the canal sometimes show what appears to be a proliferation of their nuclei, and collections of inflammatory cells may be in evidence in the ring of tissue composing the wall. The large motor nerve cells may show little or no alteration except cloudiness. Some of them, however, in acute cases may show small vacuoles in the cell protoplasm, while others may present a necrotic appearance with failure of the nucleus to take up the nuclear stains. In chronic cases which have been allowed to survive in a paralytic condition (naturally these are few in number) there is distension and fulness of the vessels and considerable thickening of the adventitia, and some of the large nerve cells are distorted and shrunken ; some may be represented by nothing more than a structureless withered mass, and there may be an increased production of the connective tissue.

" As regards the regions in which the lesions are most evident these are, generally speaking, the lumbar region and the cervical enlargement, but this is not constant. In the brain there may be nothing beyond more or less intense congestion of the small vessels, with or without small hæmorrhages, although the inflammatory changes in the cord may be acute. The meninges may be affected, but in the majority of cases examined they were not, and in most cases the cerebro-spinal fluid has been found to be practically devoid of inflammatory cells."

The natural cases of the disease dealt with in this paper were obtained from farms on which louping-ill is enzootic. All these cases occurred in the spring or early summer, during which seasons louping-ill is most prevalent. A summary of the salient points relating to the cases from which material was collected is given in Table I,

TABLE I.

Case No.	*History and Symptoms.*	*Gross Pathology.*	*Cultures from Nervous System.*	*Transmission Experiment.*
1929 Ewe 5	Fourteen days before onset of illness this ewe was removed from a " healthy " farm to a farm on which louping-ill was prevalent, where she lambed. When first noticed ill, she was salivating, showing protrusion of tongue, inco-ordination of gait, falling occasionally, but on being replaced on her feet could run a considerable distance. There were occasional periods of stupor during which the animal stood with head almost touching the ground. Temperature, 103° F. Next morning temperature 102·6° F.; Semi-comatose, but the corneal reflex still persisted; there were occasional paddling movements of limbs. Destroyed.	No macroscopic lesions other than congestion of the vessels of the pia mater and slight œdema of substance of the cord. The grey matter was conspicuous, being pink in colour.	Negative	Virus present
1930–1	Cheviot ewe hogg found lying quietly except for occasional kicking movements. Was able to stand when supported, tremors of lips and marked shaking of the head. Temperature 103·4° F. Next day the animal was semi-comatose and showed occasional kicking movements. Destroyed.	Slight congestion of the vessels of the brain and cord; cerebro-spinal fluid removed before death was clear.	Negative	Virus present
1930–2	Cheviot ewe nursing a lamb 3-4 days old. Described by shepherd as having been " in a fit " but recovered except for stiffness in gait. Next day the animal became comatose. Following day still comatose; temperature 103° F. No tremors and no kicking movements. There was slight salivation. Destroyed.	Blood clots over the surface of the cerebellum and over the surface of the thoracic and lumbar region of the cord.	Negative	Virus absent
1930–3	Cheviot ewe 4 years old, nursing twins 10 days old. Found lying unconscious, kicking violently; tremor of lips, head shaking and profuse salivation. Temperature 104·2° F. Died 20 minutes after these symptoms were observed.	Vessels of meninges were congested. A small subdural hæmorrhage in anterior thoracic region of cord.	Negative	Virus absent
1930–4	Cheviot ewe 4 years old, nursing a lamb 6 days old. Noticed staggering, falling occasionally, but able to rise and follow the other sheep. At that time she still took an interest in her lamb; about 1 hour later she isolated herself, lay down, became unconscious, kicking violently and salivating profusely. Died 10 minutes later.	Central nervous system appeared normal. No macroscopic lesions.	Negative	Virus absent

TABLE I. Continued.

Case No.	*History and Symptoms.*	*Gross Pathology.*	*Cultures from Nervous System.*	*Transmission Experiment.*
1930–5	Cheviot hogg. Seen by shepherd lying, kicking and salivating profusely. Died 24 hours after first symptoms were observed.	Post-mortem carried out 2 hours after death. Distinct congestion of vessels of brain substance and meninges. Marked œdema of the cord; the grey matter showed up prominently, being pink in colour. The cut surface projected beyond the pia mater.	Cultures made from brain substance and cord were sterile after 24 hours incubation. Not subsequently examined.	Virus present
1931–140	Blackface hogg. Showing irregularity in gait. Occasionally fell into a state of stupor, standing with nose touching the ground. When urged on it appeared bright. These symptoms continued for two days and on the morning of the third day the animal was found dead. There was evidence that paddling movements of the limbs had occurred before death.	No abnormality noticed.	Negative	Virus present
1931–153	Blackface hogg noticed dull and lagging behind the other sheep for two days. On the third day still dull; straddling gait with occasional excessive flexion in hind limbs. Later unable to rise, showing occasional convulsive movements of limbs. Temperature 102·4° F. Destroyed by bleeding.	Some excess of peritoneal fluid present. (Therefore not strictly belonging to Group 3 of M'Fadyean's classification.)	Cerebrum, no growth. Lumbar cord, Gram + coccus regarded as a contaminant.	Virus present
1931-167	Blackface hogg found unable to rise and showing paddling movements of limbs, slight salivation and tremors of lips. Temperature 100·6° F. Destroyed by bleeding.	No abnormality observed.	Negative	Virus present
1931–2	Cheviot lamb about 10 days old. Noticed staggering by shepherd. Later on same day found unable to rise; showing paddling movements of limbs and slight salivation. Temperature 102° F. A few hours afterwards was semi-comatose. Destroyed at this stage.	No gross lesions in any of the organs.	No cultures made	Virus present

HISTOLOGICAL EXAMINATION OF TISSUES FROM THE CENTRAL NERVOUS SYSTEM OF THE CASES SHOWN IN TABLE I.

Methods.

The material was fixed in formol-ammonium bromide, embedded in paraffin and stained by Delafield's hæmatoxylin and eosin and by Giemsa. In some instances alcohol fixed tissues were stained by toluidin blue to demonstrate Nissl's granules. Tissues fixed in Duboscq-Brasil or in formol-ammonium bromide and stained by Mann's method were examined for inclusion bodies.

I. Cases in which Virus was Found to be Present.

Cerebrum.—The material examined was taken from the dorso-lateral region about one inch anterior to the occipital pole.

Cases 1929/Ewe 5, 1931/1, 1930/5, 1931/140, 1931/153, 1931/167, and 1931/2 showed lesions varying in degree of intensity. These consisted of congestion of the vessels of the meninges, infiltration of round cells into the meninges, more especially around the blood vessels and more marked in the sulci than over the gyri. The vessels of the substance showed varying degrees of congestion and peri-vascular infiltration with round cells. Also there was an increase in cells scattered diffusely throughout the substance and small foci of cells were occasionally present. In Case 1930/5 an occasional polymorphonuclear leucocyte was present in the infiltrate around the vessels of the meninges. In Cases 1931/153 and 1931/2 fairly numerous polymorphonuclear leucocytes were present in the infiltrate of the meninges, and they were also found around some of the vessels of the substance. Here also in some areas of the cortex polymorphonuclear leucocytes were scattered diffusely in considerable numbers, but in other areas of the same section none could be found. When present in the cortex they were seen in some instances in very close relation to large nerve cells and were engaged in neurophagocytosis. In addition, in Case 1931/153 a few eosinophil leucocytes were present in the meningeal and perivascular infiltrates. With the exception of the two cases mentioned changes in the nerve cells were not observed.

Cerebellum.

Sections of material from the same case as described under " Cerebrum " showed congestion of the vessels of the meninges and round cell infiltration into the meninges. As in the cerebrum, this infiltration was more marked in the sulci than over the gyri, but here, as a rule, it was rather more intense than in the cerebrum with the exception of two cases (1931/153 and 1931/2) in which the converse condition obtained. In the substance there was congestion and perivascular infiltration of the vessels. These lesions were fairly

constant and were well marked. In Case 1929/Ewe 5, small foci of cells were present in the molecular layer and in Case 1931/2 foci were in evidence in the white substance. An occasional polymorphonuclear leucocyte was found in the meningeal infiltrate of Cases 1930/5 1931/140 and 1931/2.

Hurst (7) found that in experimental louping-ill of the monkey the cerebellum was far more severely affected than in other neurotropic virus diseases. He found wholesale destruction of the Purkinje cells, of which only a small proportion survived in recognisable condition. Microglial reaction was disproportionately meagre.

In our natural cases of louping-ill a variable diminution in the number of Purkinje cells occurred. Cases 1931/167 and 1931/2 showed only a few healthy cells, the large majority having completely disappeared. In these two cases also there was a marked diffuse increase in microglial cells in the molecular layer ; this was more intense close to the Purkinje cell layer. The diminution in the number of Purkinje cells in the other cases, though present, was not nearly so marked (Fig. 1). In some instances clear spaces were seen ; these probably represent the site of vanished Purkinje cells. With the exception of Case 1931/140 all showed some increase in the microglial elements in the molecular and Purkinje cells layers. This increase as a rule was most marked where the Purkinje cells were absent or few in number. In Case 1930/1 some of the Purkinje cells, particularly their processes, appeared swollen. Others which appeared necrotic had lost their regular shape, the nucleus had disappeared, and the

Fig. 1.

Section of Cerebellum, Natural Case 1930-1 ; H.E.B. × 50. Showing perivascular infiltration, cellular infiltration into the meninges and diminution in the number of the Purkinje cells.

cell cytoplasm was faintly stained. Some of these show a small number of phagocytic cells in close approximation. Lysis of the Purkinje cells by phagocytes was occasionally observed.

Fig. 2.

Section of Medulla, Natural Case 1930-1; H.E.B. × 75. Showing perivascular infiltration.

Hippocampus.

An examination of this tissue from Cases 1930/1, 1930/5, 1931/140, 1931/167 and 1931/2, revealed well marked perivascular infiltration of the vessels. In addition in Case 1930/5 a few small foci of cells were present in the substance.

Medulla.

Tissues from Cases 1930/1 and 1930/5 showed well marked perivascular infiltration around the vessels of the substance (Fig. 2). The pia mater was not infiltrated with cells. In addition in Case 1930/5 there was a slight diffuse infiltration and a few well-marked foci of cells present in the substance. Some of the foci were closely related to large nerve cells which appeared necrotic, the nucleus being indistinct, the cytoplasm poorly stained, and the cell outline irregular. The majority of the nerve cells were healthy.

Spinal Cord.

Sections were prepared from the cervical, thoracic, and lumbar segments from each case. The pial folds were infiltrated with cells in some degree. The inflammatory process appeared to be spreading

from the grey matter outwards towards the periphery of the cord, as infiltrating cells were most numerous in the depths of the pial folds close to the grey matter and became fewer in number towards the periphery. Such infiltrating cells were never observed on the surface of the cord. All cases showed varying degrees of perivascular infiltration of the vessels of the grey and white matter (Fig. 3). Cases 1929/Ewe 5, 1930/5, 1931/153 and 1931/2 showed the most marked diffuse and focal infiltration of cells into the grey matter. Some foci were apparently unrelated to vessels or nerve cells, others were seen surrounding large motor nerve cells, some of which, especially in the ventral horn, were necrotic and exhibited commencing neuronophagia.

In Cases 1930/1, 1931/140 and 1931/167 perivascular infiltration of the vessels of the grey and the white matter and infiltration into the depths of the pial folds were the most prominent features. In Cases 1931/2 and 1931/153 polymorphonuclear leucocytes were

FIG. 3.

Section of Cervical Cord, Natural Case 1930-5; H:E.B. × 75. Showing perivascular infiltration.

present in considerable numbers in the diffuse and focal cellular infiltrates, and their part in phagocytosis of nerve cells was a conspicuous phenomenon. (Fig. 4.)

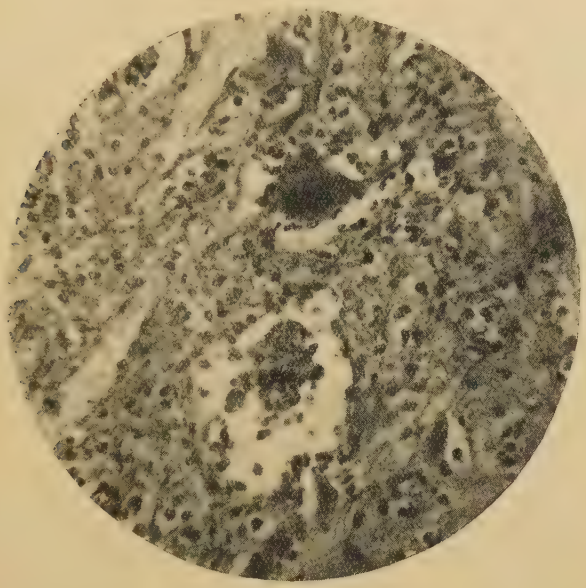

FIG. 4.
Section of Lumbar Cord, Natural Case 1931-2; H.E.B. × 240. Showing increase in microglia and neuronophagia of large nerve cells.

Changes in the Nerve Cells of the Spinal Cord.

Much variation appeared to occur in the degree of nerve cell destruction in the different cases and in different regions of the central nervous system in a particular case. As a rule, the cervical and lumbar regions suffered most severely. In no part, however, was there any extensive destruction of motor neurons, and indeed, in some cases, phagocytosis of nerve cells was not demonstrable. The nerve cells showed much variation in the different cases. In some, practically all the nerve cells appeared healthy; in others, varying proportions of the the cells showed dissolution of the Nissl granules and presented a cloudy appearance. Exceptionally the cytoplasm contained a few small vacuoles. Later stages of the nerve cell degeneration appeared to be represented by cells showing fading nuclei, poorly staining eosinophilic cytoplasm, and irregular outline. Varying degrees of neurophagocytosis could be discerned; some nerve cells were seen with only a few surrounding phagocytic cells, others were surrounded by considerable numbers. More rarely the nerve cell had completely, or almost completely, disappeared, and was replaced by a focus of phagocytes.

Spinal Ganglia.

Sections examined from all the cases except Case 1931/153 (from which ganglia were not taken for examination) showed no abnormality.

Nerves.

The sciatic and median nerves of two natural cases (1931/140 and 1931/167) were examined. In both sciatic nerves a number of round cells were present around vessels in the interfascicular tissue. The median nerve of Case 1931/140 was similarly affected, but that of Case 1931/167 was free of invading cells.

Throughout these tissues the large majority of the cells comprising the perivascular infiltrate and the infiltrate of the meninges consisted of mononuclear cells. Most of these were cells with round deeply staining nuclei surrounded by a greater or a lesser amount of cytoplasm. These resembled the lymphocytic type of blood cell. Cells with a round nucleus containing a few large fragments were present in varying, but never large, numbers. A few cells possessed polymorphic nuclei, these being frequently crescent-shaped.

In the diffuse and focal infiltrates mentioned above as occurring in the tissues, cells corresponding very closely to microglia as described by Hurst (8) were most prominent. Their nuclei were very polymorphic and appeared oval, lobed, rod-shaped, sickle-shaped, or irregular in outline. In some cases the rod-shaped type predominated. These nuclei were larger, more granular, and less intensely stained than those of the mononuclears. Mononuclear cells resembling lymphocytes were usually present although never in great numbers. Polymorphonuclear leucocytes were present in certain cases in the perivascular, meningeal, diffuse and focal infiltrations. Their distribution has already been described.

Inclusion Bodies.

The hippocampus of Cases 1931/140, 1931/167 and 1931/2 fixed in Duboscq-Brazil, and spinal ganglia from Cases 1930/1, 1930/5, 1931/140, 1931/167 and 1931/2 fixed in formal-ammonium bromide or in Duboscq-Brasil and stained by Mann's method, were examined. Inclusion bodies were found in the cytoplasm of some of the large cells of the spinal ganglia of all cases except Case 1931/2.

In some cases these inclusion bodies were numerous ; in others only a few occurred. Their size varied, the largest corresponding in size to that of the nucleolus of a ganglion nerve cell. Stained by Mann's method they were droplet like and bright pink in colour. Further study led us to the conclusion that they were not specific as we found exactly similar bodies in the spinal ganglia of sheep suffering from pathological conditions other than louping-ill. In no case were inclusion bodies found in any of the cells of the hippocampus.

II. Examination of Tissues of those Cases from which Virus was not Obtained (1930/2, 1930/3 and 1930/4).

The cerebrum and medulla, cervical, thoracic, and lumbar regions of the spinal cord showed no morbid changes resembling those found in the natural cases of louping-ill from which virus was obtained. The only abnormal feature observed was congestion of the vessels ; this varied in intensity and was not constantly present.

In the cerebellum, however, of one of the cases (1930/4) there was a distinct perivascular infiltration around a few of the vessels of the white substance. The affected vessels were localised to one area, other areas in the same section being quite normal. The sections from the cerebellum of the other two cases showed no abnormality.

Since we had demonstrated inclusion bodies in the spinal ganglia of some of the positive cases, a search was made for similar bodies in the ganglia of the negative cases. In all three negative cases similar bodies were found in even greater numbers than were present in those cases from which virus had been obtained. Reference to Table I will show that the cases in which these inclusion bodies were most numerous were adult sheep.

It will also be observed that all the cases of louping-ill in which virus was present and in which lesions of the central nervous system were demonstrated occurred in lambs or in sheep under one year of age ; the only exception to this was Case 1929/Ewe 5, which only a short time previously had been removed from a farm on which there was no history of louping-ill to one on which louping-ill was prevalent.

EXPERIMENTAL LOUPING-ILL OF THE SHEEP.

Sections of tissues from the experimentally infected sheep were prepared for examination in a similar manner to those from the natural cases.

The tissues examined were obtained from the cases summarised in Table II.

Cerebrum.

Lesions were present in the cerebrum of all cases examined and, like the lesions found in the natural cases, presented some variation in the degree of their intensity. In all cases well marked cellular infiltration into the meninges was observed. Infiltration of cells around the vessels of the substance was a constant feature and, as a rule, was intense. The degree of diffuse infiltration of cells into the substance was more variable ; in some only a slight increase in the cellular elements was noticeable ; in others this phenomenon was prominent. Foci of cells were usually absent ; when present they were never numerous. In Case 449 polymorphonuclear leucocytes were present in

TABLE II.

EXPERIMENTAL LOUPING-ILL OF THE SHEEP.

Case No.	*History and Symptoms.*	*Gross Pathology.*	*Cultures from Nervous System.*
Sheep 589	Inoculated intracerebrally with virus from previous experimental case. On the fifth and sixth days after inoculation animal showed slight dulness. On the seventh day it was found in the recumbent position, and on being approached was able to rise and managed to stagger a few yards before again falling. Destroyed by bleeding at this stage.	Liver and kidneys pale. No other gross lesions observed.	Cultures made from the brain were sterile after 2 days incubation ; not subsequently examined.
Sheep 842	Inoculated intracerebrally with virus from previous experimental case. Four days after inoculation animal slightly dull ; on fifth day still dull and tended to carry head higher than normal. On sixth day salivating ; lips, nostrils and ears twitching. On seventh day killed by bleeding.	No gross change in any of the organs.	Cultures from brain sterile after 9 days incubation.
Sheep 846	Inoculated intracerebrally with brain and spinal cord suspension from natural Case 1930/5. On sixth and seventh days after inoculation animal was dull. On eighth day profuse salivation, inability to rise, but could stand for a few seconds when raised on to its feet. Destroyed at this stage.	No gross lesions.	A coccus present in one of two tubes inoculated from brain. The other tube remained sterile.
Sheep 833	Inoculated intracerebrally with suspension of brain from experimental case. Animal noticed dull on the fifth day ; dulness more pronounced on the sixth day ; animal tended to lie, and on being raised staggered a few yards. On the seventh day unable to rise ; showed champing of the jaws and later became semi-comatose ; destroyed at this stage.	Congestion of meninges. No other gross lesions.	Cultures from brain remained sterile after 12 days' incubation.
Sheep 449	Inoculated intracerebrally with a suspension of brain from experimental case of Louping-ill in the pig. Became dull on eighth day ; semi-comatose and unable to stand on ninth day. Later on ninth day it became completely comatose and was destroyed.	The only gross lesions found were congestion of brain and cord.	Cultures from the brain remained sterile.

the meningeal and perivascular infiltrates and were also scattered diffusely in the substance. An occasional polymorphonuclear leucocyte was present in the substance of Case 846.

Cerebellum.

Examination of the cerebellum revealed varying degrees of cellular infiltration into the meninges and perivascular infiltration of the vessels of the substance. Diffuse infiltration with cells into the molecular layer was noted in Case 449 ; in Case 846 diffuse and focal infiltration occurred.

As in the natural cases a varying diminution in the number of Purkinje cells and a varying microglial reaction was noticed. Cases 449 and 842 showed very few remaining Purkinje cells ; considerable numbers of healthy Purkinje cells were, however, still present in Cases 589, 833 and 846 (Fig. 5). The increase in the microglia was not constantly proportionate to the diminution in the numbers of Purkinje cells. For instance, there was little or no microglial reaction in the molecular and Purkinje cell layers of Case 842. Quite a number of Purkinje cells in Case 589 were surrounded, and a few almost completely removed, by phagocytic cells.

FIG. 5.

Section of Cerebellum, Experimental Case 846 ; H.E.B. × 75. Showing cellular infiltration into the meninges and diminution in the number of the Purkinje cells.

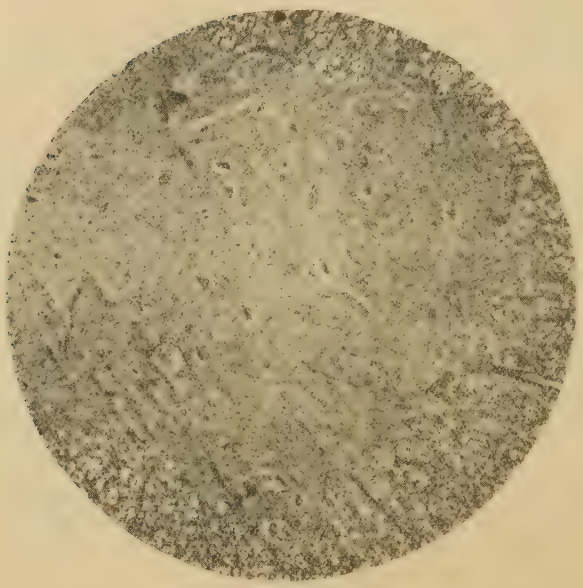

FIG. 6.

Section of Cervical Cord, Experimental Case 589; H.E.B. × 50. Showing perivascular infiltration, diffuse microglial reaction and commencing phagocytosis of nerve cells.

Hippocampus.

A well marked perivascular infiltration of the vessels of the substance constituted a feature common to all cases. In Cases 846 and 842 foci were present among the large pyramidal nerve cells; in the former case these foci consisted mainly of polymorphonuclear leucocytes, whereas in the latter they were microglial in type. Case 833 showed a few polymorphonuclear leucocytes in the perivascular infiltrate and scattered in the substance.

Medulla.

As a rule, the meninges on the surface were free of infiltrating cells. Only in one case were such cells present. Intense perivascular infiltration of the vessels of the substance was a marked feature in this region.

Varying changes in the large nerve cells were present in all cases. Cases 846, 589 and 833 showed nerve cells which were faintly stained, swollen, and vacuolated, and some were surrounded and attacked by phagocytic cells of the microglial type. These changes were most pronounced in Case 833, in which in some instances only a group of invading cells marked the site of a phagocytosed nerve cell. In this case also, one large nerve cell was noticed to contain several polymorphonuclear leucocytes in its substance, causing lysis of the cytoplasm. A few other polymorphonuclears were present scattered in the substance. There was a marked diffuse infiltration

of microglial cells among the large nerve cells in Case 589. In Case 842 no neurophagocytosis was observed, although foci of cells were present. (The medulla of Case 449 was not examined).

Spinal Cord.

In all regions examined, the meninges on the surface of the cord were free of infiltrating cells. The folds, however, of the pia mater contained a variable number of cells, these being most numerous in the depths of the folds adjoining the grey matter. Perivascular infiltration was present in a varying degree and with one exception was less marked than in the brain. The cervical region of Case 589 showed heavy infiltration into the pial folds and a well marked perivascular infiltration of the vessels of the substance (Fig. 6). Diffuse infiltration into the grey matter was present in Case 589 and diffuse and focal infiltration in Case 449. Case 589 also showed changes in the nerve cells similar to those found in the medulla of this case. The other cases showed similar nerve cell changes in less degree. Generally speaking, lesions occurred throughout, these being more marked in the cervical than in the thoracic and lumbar regions.

The types of cells in the various infiltrates in the above tissues appeared indistinguishable from those encountered in the tissues of the natural cases. The presence of polymorphonuclear leucocytes was inconstant ; these have received particular reference under the description of the tissues in which they were found.

Peripheral Nerves.

The material for this examination was obtained from two sheep (60 and 61) which had been inoculated intracerebrally with the virus of louping-ill and were showing symptoms of louping-ill when destroyed. Both cases showed lesions similar to those above described in their central nervous system.

In the sciatic and median nerves of Case 61 foci of round cells were present between the bundles of fibres (in the epineurium) and also between the individual fibres (in the endoneurium). The vagus nerve of Case 61 and the sciatic, median, and vagus nerves of Case 60 appeared normal.

Other Organs.

Liver and kidney from three experimental cases (60, 61 and 936) were examined. The only feature of outstanding note was well marked cloudy swelling of the cells of the convoluted tubules of the kidney of Case 936.

Inclusion Bodies.

The hippocampus and the spinal ganglia of four cases of experimental louping-ill in the sheep were examined in a similar way to the corresponding tissues from the natural cases.

The hippocampus did not in any case show any inclusion bodies. In the spinal ganglia of all four cases a few inclusion bodies similar to those described under the natural cases were found.

EXPERIMENTAL LOUPING-ILL OF THE PIG.

The pig was found susceptible to the virus, but the syndrome in this animal was quite different from that shown by the sheep. A generalised paralysis ensued about six days after inoculation and lasted for a week or longer with, as a rule, gradual recovery. (For details of symptoms see (i).)

Pig 457.

This pig showed commencing paralysis six days after intracerebral inoculation with the virus of louping-ill and was destroyed one day later, at which time it was unable to stand.

A post-mortem examination showed no abnormality other than congestion of the vessels of the meninges.

Cerebrum.

Sections showed well marked cellular infiltration into the meninges, and in the substance the vessels showed a very intense perivascular infiltration. Focal and diffuse infiltration of the tissues with cells was another prominent feature. A few polymorphonuclear leucocytes were present scattered in the substance and a few in the perivascular infiltrate.

Cerebellum.

There was a very intense infiltration of cells into the meninges, more marked in the sulci than over the gyri. The lesions in the substance were similar to those of the cerebrum except that here they were more pronounced. Nodules of cells were present in the Purkinje and molecular cell layers. Where the infiltration into the Purkinje cell layer was intense Purkinje cells were either obscured or absent. A few Purkinje cells were necrotic and surrounded by phagocytic cells. The large majority of the Purkinje cells were healthy.

In the cerebrum and cerebellum the majority of the infiltrating cells in the meninges and in the perivascular infiltrate were large and small mononuclear cells. In the meninges of the cerebellum, and to a less extent in the cerebrum, there was a considerable number of eosinophil leucocytes. The majority of the cells of the diffuse and focal infiltrates possessed irregular nuclei, these being round, oval, rod or sickle-shaped. The nuclei of these cells were faintly stained and contained several very small deeply staining granules. The rod-shaped type was often more deeply stained. Included in these infiltrates were lymphocytes and occasional polymorphonuclear leucocytes but eosinophil leucocytes were not observed.

Spinal Cord.

Lesions were found in the cervical, thoracic and lumbar regions of the cord. These consisted of intense infiltration with mononuclear cells into the folds of the meninges. This infiltration was most intense in the depths of the pial folds, but the process had extended outwards and a few cells had been discharged into the pial meshes on the surface. The perivascular sheaths of the vessels of both grey and white matter were markedly infiltrated with round cells, among which eosinophil leucocytes were occasionally present. There was a marked diffuse and focal infiltration of cells into the grey matter (Fig. 7). These cells were similar in type to those of the focal infiltrate in the brain. Most of the motor nerve cells appeared to be healthy ; a few were necrotic and surrounded by numerous phagocytic cells (Fig. 8.) These necrotic nerve cells were cloudy and had lost their sharp angular outline. The cytoplasm appeared homogeneous and in hæmatoxylin and eosin stained sections was eosinophilic. Nissl granules were chromatolysed. In some, the nucelus was not visible ; in others, it appeared shrunken, irregular in outline and only

FIG. 7.

Longitudinal section of Cervical Cord, Pig 457 ; Giemsa × 75. Showing diffuse and focal cellular infiltration.

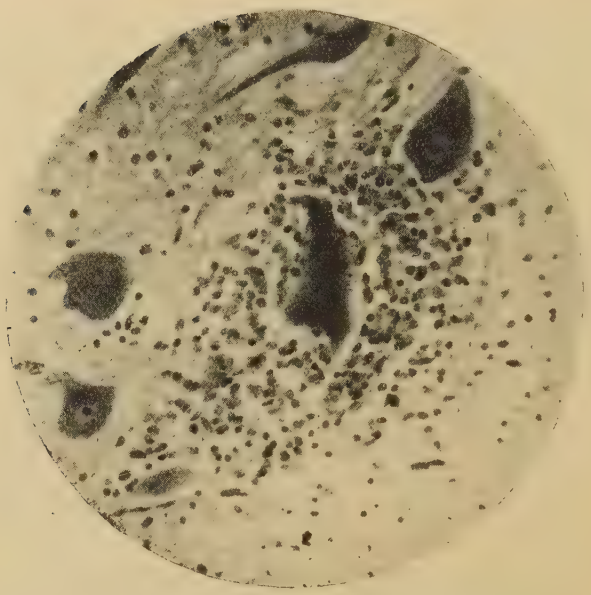

FIG. 8.

Same section as in Fig. 7 × 240. Showing necrotic nerve cell undergoing phagocytosis.

faintly stained. Nerve cells, adjoining those of their fellows which were necrotic and surrounded by numerous phagocytic cells, might appear perfectly healthy. Polymorphonuclear leucocytes were present in small numbers in the perivascular infiltrate and were also scattered in the diffuse and focal cellular infiltrates of the grey matter.

Pig 325.

This pig was inoculated intracerebrally with suspension of dried mouse-brain virus. Paralytic symptoms and inability to stand ensued on the eighth day, at which time the animal was destroyed.

In this pig the lesions were of a similar nature to those found in Pig 457. The cerebellum showed even more marked diffuse and focal infiltration into its substance and more intense infiltration into the meninges (Fig. 9). The lesions in the thoracic and lumbar cord were very slight and did not compare in their degree of intensity with the lesions in the corresponding tissues of Pig 457.

Section material was examined from two other pigs (454 and 458) which had been inoculated intracerebrally with the virus of louping-ill and had partially recovered from the experimental disease when destroyed.

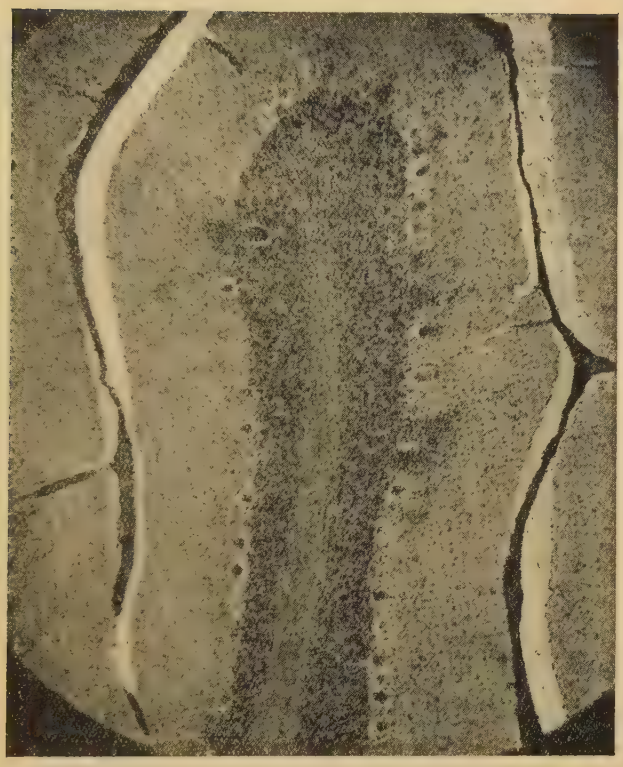

FIG. 9.

Section of Cerebellum, Pig 325; H.E.B. × 75. Showing intense cellular infiltration into meninges; focal and diffuse cellular infiltration into the molecular and Purkinje cell layers,

Pig 454.

This animal was destroyed six days after the first symptoms of paralysis made their appearance and had so far recovered as to be just able to stand. It, however, still showed marked tremblings of the body while standing.

Cerebrum.

The meninges were sparsely infiltrated with round cells. The vessels of the substance showed varying degrees of perivascular infiltration, though this was never very intense. Small foci of cells were present in the substance.

Cerebellum.

There was fairly well marked cellular infiltration into the meninges. The mononuclear type of cell predominated. A number of eosniophils and occasional polymorphonuclear leucocytes were also present.

There were also observed varying degrees of perivascular infiltration around the vessels of the substance, small foci of cells, and diffuse cellular infiltration, especially into the molecular and Purkinje cell layers. Where this reaction was most intense there was slight diminution in the numbers of Purkinje cells.

Cervical Cord.

A few of the vessels of the grey matter showed mononuclear cells in their perivascular sheaths. There was a diffuse infiltration into the grey matter with cells resembling those found in the focal and diffuse infiltrates of Pig 457. An occasional motor nerve cell was seen to be necrotic and was surrounded by phagocytic cells.

Thoracic Cord.

The lesions in the thoracic portion of the cord were similar to but less intense than those of the cervical region.

Lumbar Cord.

The lesions were similar to those of the cervical region.

Pig 458.

This pig had been paralysed for five days before it was destroyed. During that time there had been a progressive recovery and the animal was eventually able to stand and was even able to walk, although walking was performed with difficulty.

Cerebrum.

A few round cells were observed in the meninges and the perivascular sheaths of the vessels of the substance. Small foci of cells were present in the substance.

Cerebellum.

Here the lesions were similar to, although rather more intense than, those in the cerebrum.

Cervical Cord.

The lesions consisted of slight infiltration into the pial folds, a few small foci of cellular infiltration into grey matter, and slight perivascular infiltration of the vessels of the substance. No neurophagocytosis was observed.

Dorsal Cord and Lumbar Cord.

The lesions were similar to those found in cervical region.

Discussion of Cases.

Pigs 457 and 325 were destroyed at the stage when the symptoms appeared to be at their most acute phase. It will be observed that in these two cases the outstanding lesion was the marked cellular infiltration. In addition, in the cord, some of the necrotic neurons

were surrounded by large aggregations of phagocytic cells. Eosinophil leucocytes occurred in much larger numbers than were encountered in sheep.

The lesions in Pigs 454 and 458 were considerably less severe, the infiltrating cells being markedly fewer in number, and it is assumed that the stage of acute cellular infiltration in these cases had been passed. In Pig 454, the road to recovery had not entirely been cleared as neurophagocytosis could still be demonstrated. The aggregations of phagocytic cells probably represented a later stage in nerve cell destruction. In Pig 458 all the nerve cells were apparently healthy and the infiltrating cells were few in number. The tissues were presumably in a more advanced stage of repair.

In those pigs destroyed during the process of recovery a diminution in the number of nerve cells was not obvious. This was to be expected since comparatively few neurons are destroyed during the acute stage of the disease.

EXPERIMENTAL LOUPING-ILL OF THE MOUSE.

History of Cases.

The material was taken from mice inoculated intracerebrally in the course of various experiments. Those selected showed paralytic symptoms such as were regarded as typical of louping-ill in mice (2 and 3). The tissues were fixed in formol and stained by hæmatoxylin and eosin.

Sections Prepared from Four Cases.

Cerebrum and Hippocampus.

These two parts were sectioned together. Compared with the normal the changes were not pronounced. The vessels of the substance were more conspicuous, due to the increase in size and number of the endothelial elements, and also to the round cells which had infiltrated into the perivascular spaces. The latter were never very numerous and consisted of mononuclear cells in all cases examined. Mononuclears were also present in the pia mater, more especially around the vessels. In the substance of some cases there was a definite increase in the long rod-shaped microglial type of cell. This was more pronounced in some areas than in others, but it occurred in both molecular and pyramidal cell layers.

Cerebellum.

In some, the only change noticed was a congestion of vessels of the meninges and of the substance ; in others, in addition, a few round cells were present around the vessels. Some vessel walls appeared to be thickened, due to an increase in the number of endothelial and adventitial cells ; other vessels appeared normal. In one animal the Purkinje cells were greatly diminished in number. Some of the remaining cells were only faintly stained and in process of disintegration. There was no microglial reaction. The Purkinje cells in the other three cases appeared normal.

Medulla.

In this region the large nerve cells were principally affected. Some of the cells were swollen and had lost their angular outline ; in some instances one could not recognise the nucleus in the mass of cell cytoplasm. The latter, also, was almost indistinguishable from the ground substance surrounding it. (Fig. 10.) Vacuolation of the cytoplasm of such cells was often present. In other large neurons the nuclei were shrunken, had lost their chromatin network, had become angular in outline, were eccentric in position, and stained deeply with hæmatoxylin. In contrast to the deeply staining nucleus there was a faintly stained, enlarged nucleolus. Such nerve cells frequently showed small vacuoles in the cytoplasm. In no instance was neuronophagia observed. Vascular changes were slight. A few vessels showed increase in endothelial and adventitial cells, and mononuclears might or might not be present in the perivascular spaces. When present they were never in great numbers. One case showed an increase in microglial cells with elongated nuclei scattered throughout the grey matter.

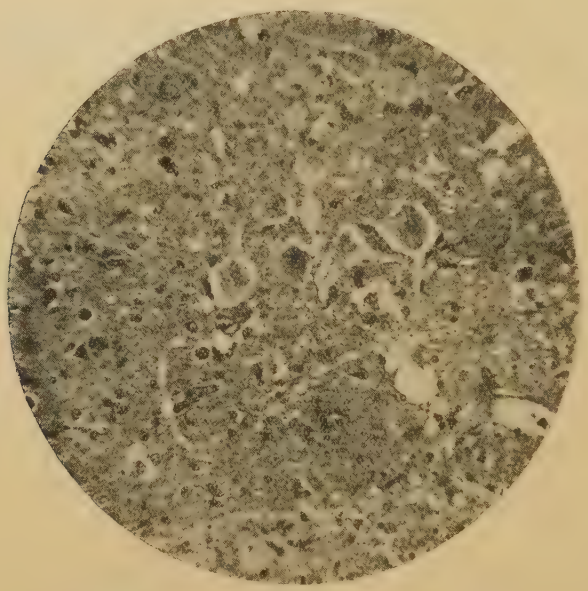

FIG. 10.

Section of Cervical Cord, Experimentally infected mouse ; H.E.B. × 240. Showing swollen necrotic nerve cells.

Spinal Cord.

All portions of the spinal cord were found to be about equally affected. No infiltrating cells could be found in the meninges on the surface of the cord, but a few were usually present in the depths of the folds of the pia mater. Only a few mononuclear cells were

encountered and these were concerned in perivascular infiltration. The vessels themselves were congested and their walls prominent from proliferation of the endothelial cells. The most striking change in the cord was represented by the damage sustained by the large motor neurons, which were affected to an even greater extent than the cells of the medulla. The large majority were in process of necrosis, their appearance being similar to that of the necrotic cells in the medulla. As in the medulla, neuronophagia was absent. There was a variable number of microglial cells with elongated nuclei scattered diffusely in the grey matter of some cases. Foci of cells were never noticed.

General Discussion.

There can be little doubt that the lesions of louping-ill in sheep, as described by M'Gowan and Rettie (5), were those which we now associate with the action of louping-ill virus. The same may be said of those cases described by Stockman (6) in which he found necrosis of neurons and infiltration of cells into the tissues and into the perivascular spaces. We do not think, however, that those cases in which Stockman found only vascular congestion with or without hæmorrhages were true louping-ill as we now recognise it. Three of our natural cases in which we found congestion of the vessels of the nervous system only, were found to represent diseased conditions other than louping-ill, in so far that material obtained from the central nervous system, when inoculated into susceptible animals, proved negative. In the tissues of our positive cases, whether these were produced naturally or experimentally, hæmorrhages were rare. When present they were small and were considered to have occurred only a short time prior to death, or to have been produced artificially during the removal of the organs *post-mortem*.

In natural and experimental cases of the disease in sheep, lesions have been found in all areas of the nervous system examined with the exception of the spinal ganglia. The vascular and interstitial changes have been of a similar character throughout the central nervous system of both groups. Compared with the corresponding tissues of cases of the natural disease, the changes in the experimental sheep have been more pronounced in the brain and less so in the cord. Necrosis of nerve cells in the cerebral cortex was observed in only two of the natural cases and in none of the experimental cases. The cerebellum was the site of constant and generally severe lesions in both the natural and experimental cases of the disease. The meningeal and perivascular infiltrations were outstanding features and a variable number of Purkinje cells were always destroyed. Irregularity of muscular action and lack of co-ordination of movement are the most prominent symptoms in the natural and experimental disease in the sheep and it is probable that such symptoms are at least partly due to the lesions in the cerebellum. Nerve cell damage in the medulla and cord is a more constant feature in sheep experimentally infected,

though it could not be said to be more severe than that seen in the cord of sheep acutely affected under natural conditions.

Greater variation in the intensity of the lesions occurs in the natural than in the experimental cases. Some natural cases show comparatively little nerve cell change. This agrees with the clinical history of the disease in the field, where it is not uncommon to meet with cases of louping-ill which recover and show no permanent disability referable to severe nerve cell damage. In other natural cases the nerve cell damage is more severe and of such a character that it is unlikely that a complete recovery would have resulted. It is the experience of flockmasters that a permanent paralysis of a group of muscles not uncommonly follows an attack of louping-ill, but an opportunity of examining the central nervous system of such cases has not yet presented itself.

Throughout the central nervous system of cases of both natural and experimental louping-ill two main types of infiltrating cells were constantly met with. In the meningeal and perivascular infiltrates mononuclear cells markedly predominated. Cells of the microglial type formed the bulk of the diffuse and focal cellular infiltrates. The distribution of polymorphonuclear leucocytes throughout the tissues was very inconstant ; in some instances, they were engaged in neurophagocytosis ; in others, they bore no positional relationship to either vessels or nerve cells. They were present in circumstances in which bacterial infection did not exist. In the pig the presence of large numbers of eosinophil leucocytes in the infiltrate of the meninges of the brain was an interesting feature.

The finding of bodies in the cells of the spinal ganglia of a case of louping-ill in an adult sheep led us to make an extended examination of the ganglia of other cases of louping-ill and of sheep dead from other disease conditions. It was found that such bodies were constantly present in considerable numbers in all adult sheep examined ; the ganglia of lambs were constantly free of such bodies, whereas in young sheep they were present only in small numbers. They appear, therefore, to be associated with the ageing of the animal and are apparently without pathological significance.

In a comparison of the lesions met with in the pig and the mouse, it will be noted that in the pig there is a severe interstitial reaction and relatively little nerve cell damage, whereas in the mouse there is very slight interstitial reaction with very severe destruction of the motor neurons. In the pig the cellular reaction to the presence of such nerve cells as were necrotic (neurophagocytosis) was a conspicuous feature ; in the mouse this phenomenon was never observed. Hurst (8) describes nerve cells obviously killed almost instantaneously which show at first little tendency towards neuronophagia. It is considered probable that destruction of nerve cells in the mouse is also rapid and that these animals die before neuronophagia has commenced. The pig and the mouse not only differ greatly in the type of lesions exhibited ; the clinical manifestations also are different. The promi-

nent symptoms in the pig are hyperæsthesia, tremors of head and limbs, and inco-ordination of movement followed by inability to stand. The animal is acutely ill for a week or longer and gradually makes an apparent complete recovery. In the mouse, on the other hand, the disease invariably terminates fatally. The tissues of the pig seem to react strongly to the presence of virus, the activity of which is eventually arrested, whereas in the mouse there is only very slight reaction, and death of the individual is due to destruction of nerve cells which are left unprotected against the spread of the infection. Taking into consideration the course of the disease in the pig, it is conceivable that the symptoms in this animal may be partly due to the heavy cellular infiltration into the nervous tissues, resulting in pressure on, and consequent irritation of, the nerve cells.

Hurst (8) is in disagreement with earlier writers who postulated that in poliomyelitis of man a primary interstitial inflammation with secondary damage to the nerve cells represents the sequence of pathological change. The lesions found in mice and pigs infected with the virus of louping-ill indicate to us that nerve cell damage in this disease is in no way proportionate to the interstitial changes. Such lesions would appear to give support to Hurst's view that the nerve cells are primarily affected by the virus and that their degeneration is not attributable to the accompanying interstitial inflammation.

Conclusions.

1.—Lesions of meningo-encephalo-myelitis have been found in all natural cases of true louping-ill. Positive diagnosis of louping-ill was determined by the presence of active louping-ill virus in the central nervous system as demonstrated by experimental inoculation of susceptible animals.

2.—No lesions of meningo-encephalo-myelitis were found in cases clinically resembling louping-ill but from which no virus was obtained.

3.—Sheep infected experimentally with the virus of louping-ill showed lesions similar to those found in the natural cases.

4.—A varying degree of destruction of the Purkinje cells of the cerebellum of both natural and experimental cases of the disease in sheep was constantly met with.

5.—Severe damage to the nerve cells of the medulla and all parts of the spinal cord, though not constantly present, often occurred in both natural and experimental cases.

6.—Specific inclusion bodies have not been demonstrated in the nervous tissues of the sheep. Intracellular bodies of a non-specific nature were found in the large nerve cells of the spinal ganglia. The tissues of the pig and the mouse have not been examined for inclusion bodies.

7.—In the pig the nervous tissues show very intense cellular infiltration and relatively little nerve cell destruction.

8.—In the mouse the principal lesion found was necrosis of the majority of the large nerve cells of the medulla and cord.

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